European Commission

JRC REFERENCE MATERIALS REPORT

The certification of the mass concentration of perfluoroalkyl substances (PFASs) in water: IRMM-428

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2015

Report EUR 27430

Joint Research Centre

European Commission
Joint Research Centre
Institute for Reference Materials and Measurements (IRMM)

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JRC97503

EUR 27430

ISBN 978-92-79-51037-3
ISSN 1831-9424

doi: 10.2787/736752

Luxembourg: Publications Office of the European Union, 20xx

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Abstract

This report describes the production of IRMM-428, a water material certified for the mass fraction of perfluoroalkyl substances (PFASs). The material was produced following ISO Guide 34:2009.
The starting material for the CRM is tap drinking water, collected in Nieuwegein, the Netherlands. The material was spiked with a mixture of PFASs, bottled and sterilised by gamma irradiation, before storage at -20 ºC .
Between unit-homogeneity was quantified and stability during dispatch and storage were assessed in accordance with ISO Guide 35:2006. Within-unit homogeneity was quantified to determine the minimum sample intake.
The material was characterised by an intercomparison among laboratories of demonstrated competence and in most cases adhering to ISO/IEC 17025. Technically invalid results were removed but no outlier was eliminated on statistical grounds only.
Uncertainties of the certified values were calculated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) and include uncertainties related to possible inhomogeneity, instability and characterisation.
The material is intended for the quality control and assessment of method performance. As any reference material, it can also be used for control charts or validation studies. The CRM is available in high density polyethylene (HDPE) bottles containing 410 mL of water material. The minimum amount of sample to be used is 100 mL.

CERTIFICATION REPORT

The certification of the mass concentration of perfluoroalkyl substances (PFASs) in water: IRMM-428

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Summary

This report describes the production of IRMM-428, a water material certified for the mass fraction of perfluoroalkyl substances (PFASs). The material was produced following ISO Guide 34:2009 [1].

The starting material for the CRM is tap drinking water, collected in Nieuwegein, the Netherlands. The material was spiked with a mixture of PFASs, bottled and sterilised by gamma irradiation, before storage at -20 ºC[1].

Between unit-homogeneity was quantified and stability during dispatch and storage were assessed in accordance with ISO Guide 35:2006 [2]. Within-unit homogeneity was quantified to determine the minimum sample intake.

The material was characterised by an intercomparison among laboratories of demonstrated competence and in most cases adhering to ISO/IEC 17025 [3]. Technically invalid results were removed but no outlier was eliminated on statistical grounds only.

Uncertainties of the certified values were calculated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) [4] and include uncertainties related to possible inhomogeneity, instability and characterisation.

The material is intended for the quality control and assessment of method performance. As any reference material, it can also be used for control charts or validation studies. The CRM is available in high density polyethylene (HDPE) bottles containing 410 mL of water material. The minimum amount of sample to be used is 100 mL.

The following values were assigned:

	Mass concentration	
	Certified value [2] [ng/L]	Uncertainty [3] [ng/L]
Perfluorobutane sulfonate (PFBS) [1]	5.5	1.4
Perfluorohexane sulfonate (PFHxS) [1]	3.6	1.0
Linear perfluorooctane sulfonate (L-PFOS) [1]	9.6	1.7
Perfluoropentanoic acid (PFPeA) [1]	4.0	1.0
Perfluorohexanoic acid (PFHxA) [1]	7.4	1.0
Perfluoroheptanoic acid (PFHpA) [1]	3.7	0.7
	Indicative value [1] [ng/L]	Uncertainty [2] [ng/L]
Perfluorononanoic acid (PFNA)	3.9	1.4

1) As defined by using liquid chromatography mass spectrometry

2) Unweighted mean value of the means of accepted sets of data, each set being obtained in a different laboratory with a method of determination including liquid chromatography mass spectrometry. Sulfonates are expressed on an anion-basis. The certified/indicative values and their uncertainties are traceable to the International System of Units (SI).

3) The uncertainty of the certified/indicative value is the expanded uncertainty with a coverage factor k = 2 corresponding to a level of confidence of about 95 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008.

[1] After concluding long-term stability of the material, IRMM-428 is shifted to a 4 ºC storage location.

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Glossary

ANOVA	Analysis of variance
b	Slope in the equation of linear regression $y = a + bx$
c	Mass concentration $c = m / V$ (mass / volume)
CI	Confidence interval
CRM	Certified reference material
EC	European Commission
EFSA	European Food Safety Authority
EN	European norm (standard)
EQS	Environmental Quality Standard
ESI	Electro spray ionisation
EU	European Union
GUM	Guide to the Expression of Uncertainty in Measurements
HDPE	High density polyethylene
ILC	Interlaboratory comparison
IRMM	Institute for Reference Materials and Measurements of the JRC
ISO	International Organization for Standardization
JRC	Joint Research Centre of the European Commission
k	Coverage factor
LC-MS	Liquid chromatography-mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
MS	Mass spectrometry
$MS_{between}$	Mean of squares between-unit from an ANOVA
MSDS	Material safety data sheet
MS_{within}	Mean of squares within-unit from an ANOVA
n	Number of replicates per unit
N	Number of samples (units) analysed
n.a.	Not applicable
n.c.	Not calculated
n.d.	Not detectable
n.r.	Not reported
p	Number of technically valid datasets
PERFOOD	Perfluorinated organics in our diet, project No. FP7-KBBE-2007-227525
PFASs	Perfluoroalkyl substances
PFBA	Perfluorobutanoic acid

PFBS	Perfluorobutane sulfonate
PFDA	Perfluorodecanoic acid
PFDoDA	Perfluorododecanoic acid
PFDS	Perfluorodecane sulfonate
PFHxA	Perfluorohexanoic acid
PFHpA	Perfluoroheptanoic acid
PFHpS	Perfluoroheptane sulfonate
PFHxS	Perfluorohexane sulfonate
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
PFPeA	Perfluoropentanoic acid
PFUnDA	Perfluoroundecanoic acid
POPs	Persistent organic pollutants
QA	Quality assurance
QC	Quality control
qNMR	Quantitative nuclear magnetic resonance
rel	Index denoting relative figures (uncertainties, etc.)
RM	Reference material
RSD	Relative standard deviation
r^2	Coefficient of determination of the linear regression
s	Standard deviation
s_{bb}	Between-unit standard deviation; an additional index "rel" is added when appropriate
$s_{between}$	Standard deviation between groups as obtained from ANOVA; an additional index "rel" is added as appropriate
SI	International System of Units
s_{meas}	Standard deviation of measurement data; an additional index "rel" is added as appropriate
SPE	Solid phase extraction
s_{within}	Standard deviation within groups as obtained from ANOVA; an additional index "rel" is added as appropriate
s_{wb}	Within-unit standard deviation
T	Temperature
t	Time
$\bar{t}$	Time elapsed at time point i
THF	Tetrahydrofuran
t_i	Mean of all t_i

$t_{\alpha, df}$	Critical *t*-value for a *t*-test, with a level of confidence of 1-α and df degrees of freedom
t_{sl}	Proposed shelf life
u	Standard uncertainty
U	Expanded uncertainty
u^*_{bb}	Standard uncertainty related to a maximum between-unit inhomogeneity that could be hidden by method repeatability; an additional index "rel" is added as appropriate
u_{bb}	Standard uncertainty related to a possible between-unit inhomogeneity; an additional index "rel" is added as appropriate
u_c	Combined standard uncertainty; an additional index "rel" is added as appropriate
u_{char}	Standard uncertainty of the material characterisation; an additional index "rel" is added as appropriate
u_{CRM}	Combined standard uncertainty of the certified value; an additional index "rel" is added as appropriate
U_{CRM}	Expanded uncertainty of the certified value; an additional index "rel" is added as appropriate
u_Δ	Combined standard uncertainty of measurement result and certified value
u_{lts}	Standard uncertainty of the long-term stability; an additional index "rel" is added as appropriate
u_{meas}	Standard measurement uncertainty
U_{meas}	Expanded measurement uncertainty
u_{rec}	Standard uncertainty related to possible between-unit inhomogeneity modelled as rectangular distribution; an additional index "rel" is added as appropriate
u_{sts}	Standard uncertainty of the short-term stability; an additional index "rel" is added as appropriate
WFD	Water Framework Directive
$\overline{x}$	Arithmetic mean of time points
x_i	Time point i
$\bar{y}$	Mean of all results
α	Significance level
Δ_{meas}	Absolute difference between mean measured value and the certified value
$v_{s,meas}$	Degrees of freedom for the determination of the standard deviation s_{meas}
$v_{MSwithin}$	Degrees of freedom of MS_{within}

1 Introduction

1.1 Background

Perfluoroalkyl substances (PFASs) are highly fluorinated aliphatic substances that contain one or more C atoms on which all the H substituents have been replaced by F atoms in such a manner that they contain the perfluoroalkyl moiety C_nF_{2n+1} (Figure 1) [5].

These human-made compounds bring together both water- and lipid- repellent properties in combination with a high chemical and thermal stability [5]. The complementarity of these properties makes PFASs and their related compounds useful for a large variety of industrial and commercial applications. Firefighting foams, textiles, products from photographic industry, semiconductors, coating additives, cleaning products and pesticides are some examples [5,6].

The high stability of the compounds, resistance to biodegradation, atmospheric photooxidation, direct photolysis and hydrolysis, results in persistency in the environment. For that reason PFASs as perfluorooctane sulfonic acid (PFOS) and its salts were recently integrated within the list of persistent organic pollutants (POPs). In the EU, their use is currently restricted by regulation [7, 8] that covers provisions regarding production, placing on the market and use of chemicals, management of stockpiles and wastes, and measures to reduce unintentional releases of POPs.

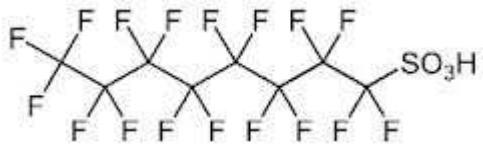


Fig.1. Example of molecular structure of a PFAS compound, linear perfluorooctane sulfonic acid, L-PFOS

The high persistence of PFASs triggers effects of bioaccumulation in the trophic chain as well. Several adverse health effects as hepatotoxicity, developmental toxicity, neurobehavioral toxicity, immunotoxicity, reproductive toxicity, lung toxicity, hormonal effects, besides a weak genotoxic and carcinogenic potential, have been demonstrated in experimental studies in animals [6,9]. Potential pathways of exposure include ingestion of food and water, the use of commercial products or inhalation from a long-range air transport [9]. Despite numerous studies are conducted to elucidate toxicological effects, levels of exposure and metabolism aspects, there is no EU legislation currently available on maximum PFAS levels in foodstuffs. Whereas for environment, the European Commission through the Water Framework Directive (WFD) recently proposed including PFOS in the list of priority hazardous substances to be monitored in the EU water bodies and set an environmental quality standard (EQS) of 0.65 ng/L for inland surface waters as well as 9.1 ng/g for biota [10].

In 2008 the European Food Safety Authority (EFSA) elaborated an opinion setting human tolerable daily intakes as 150 ng/kg and 1500 ng/kg body weight for PFAS compounds as PFOS and perfluorooctanoic acid (PFOA) respectively [11]. More recently, a dietary intake estimation conducted by EFSA concluded with a high frequency of non-quantifiable results (<LOQ) preventing the calculation of a more realistic dietary exposure. Consequently EFSA recommended the improvement on the sensitivity of analytical methods as a tool to increase the proportion of quantified results and thereby the reliability of exposure assessments for PFASs [6].

Over the last ten years a number of international interlaboratory studies have been sequentially conducted in the frame of EU funded projects to assess the overall performance of laboratories on the analysis of PFASs [12, 13, 14]. The outcome of the exercises allowed a gradual improvement of the analytical results after identification of critical factors within the process. The use of well-defined calibrants, the use of mass-labeled internal standards or minimising matrix effects were named as key elements [12]. Still comparability of results between different laboratories may remain challenging, e.g. when different sources of standards are employed.

Analytical method validation requires the assessment of performance characteristics such as precision and trueness. The most appropriate tool for evaluating accuracy is the use of certified reference materials (CRMs).

To improve comparability and harmonisation of analytical results, the production of CRMs for PFASs was included as part of the activities for the European research project PERFOOD (Perfluorinated Organics in Our Diet, No. FP7-KBBE-2007-227525). In this context, the IRMM was requested to produce two CRMs for perfluoroalkyl substances (PFASs) in fish tissue and drinking water respectively. The task was performed in close collaboration with the Institute for Environmental Studies (IVM), VU University, Amsterdam, The Netherlands.

1.2 Choice of the material

The base material employed for CRM IRMM-428 was drinking water collected from a regular tap in Nieuwegein, The Netherlands. After a pre-screening revealing low or non-quantifiable levels of PFASs (data not shown), the drinking water was spiked with a mixture of PFASs to reach target levels around 10-20 ng/L per individual compound. The concentration levels were set as to be in a low range of concentrations although still detectable with a guaranteed level of confidence (> LOQ). After processing and sterilization by gamma irradiation, the bottles were stored at -20 °C[2].

1.3 Design of the project

The project was designed in collaboration between IRMM and the Institute for Environmental Studies (IVM), VU University, The Netherlands, under the auspices of the PERFOOD European project.

A laboratory intercomparison was planned for the characterisation of the candidate reference material involving a number of expert laboratories participating in the PERFOOD consortium. The number of laboratories was found to be critically low for the success of the project. Therefore IRMM selected four additional laboratories ISO 17025 accredited in the relevant field, to take part in the material certification campaign. The laboratories were instructed to apply their own validated analytical methodology for the determination of PFASs. Together with the samples of IRMM-428, the laboratories received ampoules containing individual solutions of two of the target compounds, PFOA and perfluorodecanoic acid (PFDA), for calibration purposes (see description in Section 7).

[2] IRMM-428 was transferred to a 4 °C storage temperature according to the conclusions from the long-term stability studies.

2 Participants

2.1 Project management and evaluation

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE
(accredited to ISO Guide 34 for production of certified reference materials, BELAC No. 268-RM)

Institute for Environmental Studies[3] (IVM), VU University, Amsterdam, NL

2.2 Processing

KWR Watercycle Research Institute[3], Nieuwegein, NL
(accredited to ISO/IEC 17043 accreditation RvA R005)

With the assistance of European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE
(accredited to ISO Guide 34 for production of certified reference materials, BELAC No. 268-RM)

2.3 Homogeneity study

Fraunhofer Institute for Process Engineering and Packaging[3], IVV, Freising, DE
(measurements under the scope of ISO/IEC 17025 accreditation DAkkS D-PL-11140-04-00)

2.4 Stability study

Fraunhofer Institute for Process Engineering and Packaging[3], IVV, Freising, DE
(measurements under the scope of ISO/IEC 17025 accreditation DAkkS D-PL-11140-04-00)

Institute for Environmental Studies[3] (IVM), VU University, Amsterdam, NL
(measurements under the scope of ISO/IEC 17025 accreditation Dutch Accreditation Council, L476)

2.5 Characterisation

3M Company- Environmental Laboratory, Maplewood MN, USA
(measurements under the scope of ISO/IEC 17025 accreditation American Association for Laboratory Accreditation, certificate number 2052.01)

AXYS Analytical Services Ltd., Sidney B.C., Canada
(measurements under the scope of ISO/IEC 17025 accreditation Canadian Association for Laboratory Accreditation Inc. CALA, A2637)

Department of Applied and Environmental Sciences[3], Stockholm University, SE
(measurements under the scope of ISO/IEC 17025 accreditation SWEDAC, 11-2501-51.1295)

Eurofins GfA Lab Service GmbH, Hamburg, DE
(measurements under the scope of ISO/IEC 17025 accreditation Certificate DAP-PL-1053.99)

Fraunhofer Institute for Process Engineering and Packaging[3], IVV, Freising, DE
(measurements under the scope of ISO/IEC 17025 accreditation DAkkS D-PL-11140-04-00)

Institute for Biodiversity and Ecosystem Dynamics-Earth Surface Science[3] (IBED-ESS), University of Amsterdam, NL

Institute for Environmental Studies[3] (IVM), VU University, Amsterdam, NL
(measurements under the scope of ISO/IEC 17025 accreditation Dutch Accreditation Council, L476)

Institute of Chemical Technology[3], Prague, CZ
(measurements under the scope of ISO/IEC 17025 accreditation Czech Accreditation Institute, No. 319/2009)

[3] Laboratory associated to PERFOOD consortium

KWR Watercycle Research Institute[3], Nieuwegein, NL
(measurements under the scope of ISO/IEC 17025 accreditation Dutch Accreditation Council, L479)

Norwegian Institute for Air Research[3], Tromso, NO
(laboratory under the scope of ISO/IEC 17025 accreditation Norsk Akkreditering, TEST 008)

VITO, Mol, BE
(measurements under the scope of ISO/IEC 17025 accreditation BELAC nr. 045-TEST)

Federal Institute for Materials, Research and Testing, BAM, Berlin, DE (qNMR analysis)
(measurements under the scope of ISO/IEC 17025 accreditation DAP-PL-2614.14)

3 Material processing and process control

3.1 Origin of the starting material

The material employed as matrix was obtained from a tap providing drinking water, located in Nieuwegein, The Netherlands. Preliminary tests performed by LC-MS/MS indicated low or non-quantifiable levels of PFASs in the drinking water (results not shown). Therefore the water was spiked with a mixture of selected PFASs.

3.2 Processing

Drinking water (650 L) was obtained and processed at the Watercycle Research Institute, Nieuwegein, The Netherlands.

Ten mL of 0.25 -0.62 µg/mL of individual PFASs in methanol (Wellington Laboratories Inc., Canada) was used as spiking material (Table 1 details the composition and Annex A includes the certificate of analysis). The spiking process was performed by step-wise dilution. First the spiking solution was added to a portion of 10 L of water in a volumetric flask. The mixture was stirred for 30 min before the content of the flask was poured into a 20 L container. The flask was rinsed with 10 L of water and added to the same container. Subsequently the 20 L of spiked water were added to a stainless steel barrel. Rinsing of the 20 L container was conducted to ensure quantitative transfer of the spiked PFASs into the barrel. After filling the barrel up to a volume of 650 L, the total bulk was stirred for three hours. At four different times during the filling, samples were collected from the tap water used for filling. Additionally an aliquot of the spiked material (410 mL) was taken out of the barrel and sent to IVM for determination of PFOS, PFOA and PFDA mass concentration levels in the spiked water, as part of the processing control.

The remaining water was stored without stirring until further processing, and the container closed to avoid any losses. On the next day, once the analytical results confirmed PFASs levels in the material and met the target mass concentrations, stirring was started about 1 hour prior to bottles filling. High density polyethylene (HDPE) Nalgene bottles with a polypropylene lid (Nalgene-Mouth Sterile HDPE bottles, 500 mL, Art no Nalgene 342089-0016), stable for irradiation, were filled with 410 mL (+/- 5 mL) of the spiked water, under constant stirring. The units processed were labelled immediately after filling/dispensing, strictly following the filling sequence. The reason for filling 410 mL was to accommodate for the expansion of the water after freezing, to avoid bottles breakage.

The filled and labelled bottles were sterilized by gamma irradiation at 25 kGy (Isotron Nederland B.V., Etten-Leur, The Netherlands) to avoid the growth of fungi or microorganisms. After sterilization, the bottles were transported to IRMM at room temperature. At IRMM, bottles were subsequently stored at -20°C.

Table 1. Composition of PFASs spiking solution as provided by the manufacturer (µg/mL, ± 5 % in methanol/water (< 1%)) (Annex A).

Target Compound	Abbreviation	Concentration (µg/mL)
Perfluorobutanoic acid	PFBA	0.40
Perfluoropentanoic acid	PFPeA	0.25
Perfluorohexanoic acid	PFHxA	0.50
Perfluoroheptanoic acid	PFHpA	0.25
Perfluorooctanoic acid	PFOA	0.50
Perfluorononanoic acid	PFNA	0.25
Perfluorodecanoic acid	PFDA	0.25
Perfluoroundecanoic acid	PFUnDA	0.25
Perfluorododecanoic acid	PFDoDA	0.25
Potassium perfluoro-1-butane sulfonate	L-PFBS	0.35*
Sodium perfluoro-1-hexane sulfonate	L-PFHxS	0.24*
Sodium perfluoro-1-heptane sulfonate	L-PFHpS	0.24*
Sodium perfluoro-1-octane sulfonate	L-PFOS	0.62*
Sodium perfluoro-1-decane sulfonate	L-PFDS	0.24*

*Expressed in µg/mL of the anion

3.3 Process control

Process control consisted on preliminary analytical measurements for ensuring suitable mass concentration levels of target PFASs in the spiked water material (results not shown). The data obtained ensured that the process control was adequate.

4 Homogeneity

A key requirement for any reference material is the equivalence between the various units. In this respect, it is relevant whether the variation between units is significant compared to the uncertainty of the certified value. In contrast to that it is not relevant if the variation between units is significant compared to the analytical variation. Consequently, ISO Guide 34 [1] requires RM producers to quantify the between unit variation. This aspect is covered in between-unit homogeneity studies.

The within-unit inhomogeneity does not influence the uncertainty of the certified value when the minimum sample intake is respected, but determines the minimum size of an aliquot that is representative for the whole unit. Quantification of within-unit inhomogeneity is therefore necessary to determine the minimum sample intake.

4.1 Between-unit homogeneity

The between-unit homogeneity was evaluated to ensure that the certified values of the CRM are valid for all units of the material, within the stated uncertainty.

The number of selected units corresponds to approximately the cubic root of the total number of the produced units. The 14 units were selected using a random stratified sampling scheme covering the whole batch for the between-unit homogeneity test. For this, the batch was divided into 14 groups (with a similar number of units) and one unit was selected randomly from each group. Three independent samples were taken from each selected unit, and analysed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Briefly, water samples were prepared by solid phase extraction (SPE) employing Waters OASIS Wax 150 mg SPE cartridges. After conditioning with methanol and water, samples were loaded into the cartridge and followed by a washing step with water/methanol (50/50, v/v). PFASs were eluted with 5 mL 1 % NH_4 in methanol. The eluent containing PFASs was concentrated to dryness, reconstituted in water/methanol (50/50, v/v) and injected into the LC system. Measurements were performed under repeatability conditions and in a randomised manner to be able to separate a potential analytical drift from a trend in the filling sequence. Reporting of results of analysis for L-PFOS, PFOA and PFDA was mandatory while reporting of any other PFASs was done on voluntary bases. The evaluation of a first set of results (not shown) revealed poor repeatability. The sample handling procedure prior to analysis was identified as a critical factor during the process. Consequently the data was discarded and an additional set of 14 samples selected as described above were analysed comprising a specific sample handling protocol step in the procedure. Basically after thawing, water bottles were both ultrasonicated for ten minutes and shaken for one hour, prior to sampling. At the moment of sampling internal standards were added as well. The analytical results obtained are shown as Tables in Annex B.

Regression analyses were performed to evaluate potential trends in the analytical sequence as well as trends in the filling sequence. No trends in the filling sequence or the analytical sequence were visible for the majority of PFASs tested. Filling trends were detected for PFOA, PFNA and PFBS at a 99 % confidence level and additionally for PFHxS at a confidence level of 95 %. The trend corresponding to the analytical sequence of PFBA was statistically significant at a confidence level of 95 %. A single outlying result for PFNA during the analysis of one single replicate caused the same effect on the mean value for that particular bottle as well as influenced the significance of the trend in the filling sequence. Whereas for PFPeA one individual result was as well identified as outlier. The outliers were retained for the evaluation since no technical reason could be found for excluding those particular results.

Quantification of between-unit inhomogeneity was accomplished by analysis of variance (ANOVA), which can separate the between-unit variation (s_{bb}) from the within-unit variation

(s_{wb}). The latter is equivalent to the method repeatability if the individual samples are representative for the whole bottle.

Evaluation by ANOVA requires unit means which follow at least a unimodal distribution and results for each unit that follow unimodal distributions with approximately the same standard deviations. Distribution of the unit means was visually tested using histograms and normal probability plots. Minor deviations from unimodality of the individual values do not significantly affect the estimate of between-unit standard deviations. The results of all statistical evaluations are given in Tables 2 and 3.

Table 2: Results of the statistical evaluation of the homogeneity studies at 99 % confidence level

Measurand	Trends (before correction)		Outliers		Distribution	
	Analytical sequence	Filling sequence	Individual results	Unit means	Individual results	Unit means
L-PFOS	no	no	none	none	normal	normal
PFOA	no	yes	none	none	normal	normal
PFDA	no	no	none	none	normal	normal
PFBA	no [1]	no	none	none	normal	normal
PFPeA	no	no	one	none	normal	normal
PFHxA	no	no	none	none	normal	normal
PFHpA	no	no	none	none	normal	normal
PFNA	no	yes	one	one	normal	normal
PFBS	no	yes	none	none	normal	normal
PFHxS	no	no[1]	none	none	normal	normal

[1]Trend statistically significant at a 95 % confident level

One has to bear in mind that $s_{bb,rel}$ and $s_{wb,rel}$ are estimates of the true standard deviations and therefore subject to random fluctuations. Therefore, the mean square between groups ($MS_{between}$) can be smaller than the mean squares within groups (MS_{within}), resulting in negative arguments under the square root used for the estimation of the between-unit variation, whereas the true variation cannot be lower than zero. In this case, u^*_{bb}, the maximum inhomogeneity that could be hidden by method repeatability, was calculated as described by Linsinger *et al.* [15]. u^*_{bb} is comparable to the limit of detection of an analytical method, yielding the maximum inhomogeneity that might be undetected by the given study setup.

Method repeatability ($s_{wb,rel}$), between–unit standard deviation ($s_{bb,rel}$) and $u^*_{bb,rel}$ were calculated as:

$$s_{wb,rel} = \frac{\sqrt{MS_{within}}}{\bar{y}} \qquad \textbf{Equation 1}$$

$$s_{bb,rel} = \frac{\sqrt{\frac{MS_{between} - MS_{within}}{n}}}{\bar{y}} \qquad \textbf{Equation 2}$$

$$u^*_{bb,rel} = \frac{\sqrt{\frac{MS_{within}}{n}}\sqrt[4]{\frac{2}{v_{MSwithin}}}}{\bar{y}} \qquad \textbf{Equation 3}$$

MS_{within} mean square within a unit from an ANOVA

$MS_{between}$ mean squares between-unit from an ANOVA

$\bar{y}$ mean of all results of the homogeneity study

n mean number of replicates per unit

$v_{MSwithin}$ degrees of freedom of MS_{within}

However, a different approach was adopted for PFNA for which one outlying unit means was detected. In these cases between-unit inhomogeneity was modelled as a rectangular distribution limited by the largest outlying unit mean, and the rectangular standard uncertainty of homogeneity was estimated by:

$$u_{rec} = \frac{\left|outlier - \bar{y}\right|}{\sqrt{3} \cdot \bar{y}} \qquad \textbf{Equation 4}$$

$\bar{y}$ mean of all results of the homogeneity study

It should be mentioned that the outlying unit means are a result of presence of outlying individual values and do not necessarily reflect the real distribution of these compounds in the material.

When a trend in the filling sequence was significant at least at 99 % confidence level, the uncertainty was assessed in a different way. This applies for PFBS and PFOA. The same approach is applied to PFHxS, showing a trend at 95 % confidence level. Here, u_{rec} was estimated using a rectangular distribution between the highest and lowest unit mean. The corrected uncertainty in those cases where there was a significant trend in the filling sequence is given in:

$$u_{rec} = \frac{\left|highest\ result - lowest\ result\right|}{2 \cdot \sqrt{3} \cdot \bar{y}} \qquad \textbf{Equation 5}$$

The homogeneity study showed no outlying unit means or trends in the filling for six out of the ten PFASs tested. Therefore in those cases the between-unit standard deviation can be used as estimate of u_{bb}. As u^*_{bb} sets the limits of the study to detect inhomogeneity, the larger value of s_{bb} and u^*_{bb} is adopted as uncertainty contribution to account for potential inhomogeneity.

For PFNA one outlying unit mean was found as well as trends in the filling sequence for PFBS, PFOA and PFHxS. However, taking these extreme values into account, the inhomogeneity as quantified as u_{rec} is in most cases still sufficiently small to make the material useful. Therefore, u_{rec} was used as estimate of u_{bb}.

Table 3: Results of the homogeneity study for PFASs in IRMM-428

Measurand	$s_{wb,rel}$ [%]	$s_{bb,rel}$ [%]	$u^*_{bb,rel}$ [%]	$u_{rec,rel}$ [%]	$u_{bb,rel}$ [%]
L-PFOS	2.86	n.c.[1)]	0.85	n.a.	0.85
PFOA	1.51	0.76	0.45	1.11	1.11
PFDA	9.28	2.93	2.77	n.a.	2.93
PFBA	3.04	1.69	0.91	n.a.	1.69
PFPeA	4.10	2.76	1.22	n.a.	2.76
PFHxA	2.84	1.36	0.85	n.a.	1.36
PFHpA	5.04	3.08	1.51	n.a.	3.08
PFNA	9.18	3.31	2.74	9.64	9.64
PFBS	3.62	4.47	1.08	4.84	4.84
PFHxS	4.92	3.28	1.47	4.37	4.37

[1)] cannot be calculated as $MS_{between} < MS_{within}$

4.2 Within-unit homogeneity and minimum sample intake

The within-unit homogeneity is closely correlated to the minimum sample intake. Due to this correlation, individual aliquots of a material will not contain the same amount of analyte. The minimum sample intake is the minimum amount of sample that is representative for the whole unit and thus can be used in an analysis. Sample sizes equal or above the minimum sample intake guarantee the certified value within its stated uncertainty.

The water material is a solution and is not expected to have any relevant inhomogeneity. Measures were taken to avoid agglomeration and to keep the molecules evenly distributed in the solution during processing. Furthermore, a protocol for sampling handling prior to analysis is provided. The assumption was confirmed by the homogeneity, stability and characterisation studies, where sample intakes as low as 100 mL were found to give acceptable repeatability, demonstrating that there is no intrinsic inhomogeneity or contamination at a sample intake of 100 mL. During characterisation, the smallest sample intake that still yielded results with acceptable accuracy to be included in the respective studies was 50 mL, however ≥ 100 mL was employed by most laboratories (Annex F, Table 1). Therefore the minimum sample intake was set to 100 mL.

5 Stability

Time, temperature and light were regarded as the most relevant influences on stability of the materials. Materials are stored and dispatched in the dark, thus eliminating practically the possibility of degradation by light. Additionally the material was sterilized by γ-irradiation to eliminate microbial growth. Therefore, only the influences of time and temperature needed to be investigated.

Stability testing is necessary to establish conditions for storage (long-term stability) as well as conditions for dispatch to the customers (short-term stability). During transport, especially in summer time, temperatures up to 60 °C could be reached and stability under these conditions must be demonstrated if transport at ambient temperature will be applied.

The stability studies were carried out using an isochronous design [16]. In that approach, samples are stored for a certain time at different temperature conditions. Afterwards, the samples are moved to conditions where further degradation can be assumed to be negligible (reference conditions). At the end of the isochronous storage, the samples are analysed simultaneously under repeatability conditions. Analysis of the material (after various exposure times and temperatures) under repeatability conditions greatly improves the sensitivity of the stability tests.

5.1 Short-term stability study

For the short-term stability study, samples were stored at -20 °C, 4 °C, 18 °C and 60 °C for 0, 1, 2 and 4 weeks (at each temperature). The reference temperature was set to -70 °C. Two units per storage time were selected using a random stratified sampling scheme. From each unit, three samples were measured by LC-MS/MS as described in the homogeneity section. The measurements were performed under repeatability conditions, and in a randomised sequence to be able to separate a potential analytical drift from a trend over storage time.

The obtained data were evaluated individually for each temperature. The results were screened for outliers using the Grubbs test. One outlying individual result was found for eight compounds at 4 ºC, all of them corresponding to the analysis of the same replicate, indicating a possible anomaly specific to the particular sample. A similar effect occurred for studies at 18 ºC and at -20 ºC, with single outliers for five and two compounds respectively corresponding to the results of the same subsamples (Table 4). However as no technical reason for the outliers could be found, all data were retained for statistical analysis. One outlier was additionally detected for PFHxA at 60 ºC as well retained during evaluation.

Furthermore, the data were evaluated against storage time and regression lines of mass concentration versus time were calculated. The slopes of the regression lines were tested for statistical significance (loss/increase due to shipping conditions). For all compounds, the slopes of the regression lines were not significantly different from zero (on 99 % confidence level) at any of the temperatures tested, with the exception of PFPeA stored at 60 ºC. Despite the absence of significant trends, the sensitivity of this test was rather limited due to large variance for the results obtained for all the temperatures assessed. This results in an increased estimation of the uncertainty contributions for a potential degradation of the samples during transport.

The results of the measurements are shown in Annex C. The results of the statistical evaluation of the short-term stability are summarised in Table 4.

No outliers were detected that could be justified for technical reasons for most analytes at -20 ºC. Therefore all outliers were retained for the estimation of u_{sts}. None of the trends was statistically significant on a 99 % confidence level for any of the temperatures except for PFPeA at 60 ºC.

The material shall be shipped frozen[4]. Dry-ice was selected as most suitable transport condition to discard any potential situation of exposure to higher temperatures, where a trend was detected for one of the PFAS compounds.

[4] Preliminary tests performed during the selection process for the container material (results not shown), indicated no significant variation on the PFASs concentration in the water for up to three freezing/defrosting cycles of the material.

Table 4: Results of the short-term stability tests

Measurand	Number of individual outlying results (retained)				Significance of the trend on a 99% confidence level			
	-20 ºC	4 ºC	18 ºC	60 ºC	-20 ºC	4 ºC	18 ºC	60 ºC
L-PFOS	none	none	one	none	no	no	no	no
PFOA	none	one	one	none	no	no	no	no
PFDA	none	none	none	none	no	no	no	no
PFBA	one	one	none	none	no	no	no	no
PFPeA	one	one	none	none	no	no	no	*yes*
PFHxA	none	one	none	one	no	no	no	no
PFHpA	none	one	one	none	no	no	no	no
PFNA	none	one	one	none	no	no	no	no
PFBS	none	one	none	none	no	no	no	no
PFHxS	none	one	one	one	no	no	no	no

5.2 Long-term stability study

For the long-term stability study, samples were stored at -20 °C, 4 °C and 18 °C for 0, 8, 16 and 24 months (at each temperature). The reference temperature was set to -70 °C. Two units per storage time were selected using a random stratified sampling scheme. From each unit, three samples were measured by LC-MS/MS. The measurements were performed under repeatability conditions in a random sequence to be able to separate any potential analytical drift from a trend over storage time. The methodology employed included SPE for sample extraction followed by LC-MS/MS. Briefly, after sampling, internal standards were added. An SPE cartridge (OASIS wax, Waters) was conditioned with 0.1 % NH_4OH in methanol, followed first by methanol and then by water. The sample was subsequently transferred through the cartridge and washed with NH_4COOH at pH 4 and tetrahydrofuran (THF)/methanol, followed by elution with 0.1 % NH_4OH in methanol. After extract pre-concentration and reconstitution into water/methanol (1:1 v/v), the purified extract was injected into the LC-MS/MS.

As during previous studies, reporting of results was mandatory for L-PFOS, PFOA and PFDA. The analysis of any other PFASs was optional. The obtained data were evaluated individually for each temperature. The results were screened for outliers using the single and double Grubbs test. Except for PFPeA, no technically unexplained outlier was observed at any of the temperatures tested (Table 5).

Furthermore, the data were plotted against storage time and linear regression lines of mass concentration versus time were calculated. The slope of the regression lines was tested for statistical significance (loss/increase due to storage conditions). For all PFASs, the slopes of the regression lines were not significantly different from zero (on 99 % confidence level) for -20 ºC, 4 ºC and 18 °C.

The results of the long term stability measurements are shown in Annex D. The results of the statistical evaluation of the long-term stability study are summarised in Table 5.

Table 5: Results of the 24-month long-term stability tests.

PFASs in IRMM-428	Number of individual outlying results			Significance of the trend on a 99% confidence level		
	-20 ºC	4 ºC	18 ºC	-20 ºC	4 ºC	18 ºC
L-PFOS	none	none	none	no	no	no
PFOA	none	none	none	no	no	no
PFDA	none	none	none	no	no	no
PFBA[1]	none	none	none	no	no	no
PFPeA[1]	one	one	none	no	no	*yes*
PFHxA	none	none	none	no	no	no
PFHpA	none	none	none	no	no	no
PFNA	none	none	none	no	no	no
PFBS	none	none	none	no	no	no
PFHxS	none	none	none	no	no	no
PFUnDA	none	none	none	no	no	no

[1]Data not available for a 24-month long-term stability study. The evaluation in this case is based on existing results obtained from an eight-month long-term stability study (PFBA) or from the pooled and normalised data from an eight- and twelve-month long-term stability studies (PFPeA) .

None of the trends was statistically significant at a 99 % confidence level for any of the temperatures, with the exception of PFPeA at 18 ºC. Therefore IRMM-428 was transferred to a storage temperature at 4 °C.

5.3 Estimation of uncertainties

Due to the intrinsic variation of measurement results, no study can rule out degradation of materials completely, even in the absence of statistically significant trends. It is therefore necessary to quantify the potential degradation that could be hidden by the method repeatability, i.e. to estimate the uncertainty of stability. This means, even under ideal conditions, the outcome of a stability study can only be "degradation is 0 ± x % per time".

Uncertainties of stability during dispatch and storage were estimated as described in [17] for each analyte. For this approach, the uncertainty of the linear regression line with a slope of zero is calculated. The uncertainty contributions u_{sts} and u_{lts} are calculated as the product of the chosen transport time/shelf life and the uncertainty of the regression lines as:

$$u_{sts,rel} = \frac{RSD}{\sqrt{\sum (t_i - \bar{t})^2}} \cdot t_{tt}$$ **Equation 6**

$$u_{lts,rel} = \frac{RSD}{\sqrt{\sum (t_i - \bar{t})^2}} \cdot t_{sl}$$ **Equation 7**

RSD	relative standard deviation of all results of the stability study
t_i	time elapsed at time point *i*
$\bar{t}$	mean for all t_i
t_{tt}	chosen transport time (1 week at -20 ºC)
t_{sl}	chosen shelf life (24 months at 4 ºC)

The following uncertainties were estimated:

- $u_{sts,rel}$, the uncertainty of degradation during dispatch. This was estimated from the stability studies at -20 °C. The uncertainty describes the possible change during a dispatch at -20 °C lasting for one week.
- $u_{lts,rel}$, the stability during storage. This uncertainty contribution was estimated from the stability studies at 4 °C. The uncertainty contribution describes the possible degradation during a 24-month storage at 4 °C.

The results of these evaluations are summarised in Table 6.

Table 6: Uncertainty of stability during dispatch and storage. $u_{sts,rel}$ were calculated for a temperature of -20 °C and 1 week; $u_{lts,rel}$ was calculated for a storage temperature of 4 °C and 24 months.

PFASs IRMM-428	$u_{sts,rel}$ [%]	$u_{lts,rel}$ [%]
L-PFOS	3.03	6.68
PFOA	3.04	8.51
PFDA	2.81	10.19
PFBA	2.11	3.88[1]
PFPeA	2.42	9.59[1]
PFHxA	2.57	4.37
PFHpA	3.70	6.30
PFNA	3.24	11.73
PFBS	2.51	12.83
PFHxS	2.69	10.01
PFUnDA	n.a.	16.13

[1]Estimated from existing data of an eight-month long-term stability study (PFBA) or from the pooled and normalised data from an eight- and twelve-month long-term stability studies (PFPeA)

No significant degradation during dispatch was observed even at 18 °C.

After the certification campaign, the material will be subjected to IRMM's regular stability monitoring programme to control its further stability.

6 Characterisation

The material characterisation is the process of determining the property values of a reference material.

The material characterisation was based on an intercomparison of results from expert laboratories, i.e. the mass concentrations of PFASs in the material were determined in different laboratories that applied different measurement procedures to demonstrate the absence of a measurement bias. Due to the nature of the analytes however, all participants used liquid chromatographic methods for the measurements. This approach aims at randomisation of laboratory bias, which reduces the combined uncertainty.

6.1 Selection of participants

Seven laboratories participating in the PERFOOD project consortium took part in the characterisation campaign of the material. Four additional laboratories were selected by IRMM based on criteria that comprised both technical competence and quality management aspects. Each participant was enquired about their quality system and requested to deliver documented evidence of its laboratory proficiency in the field of PFASs measurements in relevant matrices by submitting results for intercomparison exercises and/or details on their methods validation. Having a formal accreditation was not mandatory, but meeting the requirements of ISO/IEC 17025 was obligatory. Where measurements are covered by the scope of accreditation, the accreditation number is stated in the list of participants (Section 2).

6.2 Study setup

Each laboratory received 3 units of the candidate CRM and was requested to provide 6 independent results, 2 per unit. The units for the candidate material characterisation were selected using a random stratified sampling scheme and covered the whole batch. The sample preparations and measurements had to be spread over three days to ensure intermediate precision conditions. An independent calibration was performed for each day of analysis.

Besides a protocol for sample handling, the laboratories were provided with independent solutions of PFOA and PFDA (2.5 µg/mL in methanol) for calibration purposes. Neat crystals employed for the individual calibration solutions preparation (Chiron AS, Trondheim, NO) were analysed by qNMR for purity assessment (Annex E).

All laboratories were requested to submit results for L-PFOS, PFOA and PFDA whereas the reporting of additional PFASs was optional (Table 1). Mass concentration for sulfonates was requested as anion basis.

6.3 Methods used

Most of the methods applied for characterisation involved sample extraction and clean-up with SPE followed by LC separation on chromatographic columns having C18 or a fluorinated stationary phase. Detection was performed by mass spectrometry using negative ionisation.

All methods and MRM transitions used during the characterisation study are summarised in Annex F (Tables 1 and 2, respectively). The laboratory code (e.g. L01) is a random number and does not correspond to the order of laboratories provided in Section 2. The lab-method code consists of a number assigned to each laboratory (e.g. L01).

6.4 Evaluation of results

The characterisation campaign resulted in a maximum of 11 datasets per PFAS compound. All individual results of the participants, grouped per measurand, are displayed in tabular and graphical form in Annex G. Laboratory with assigned code L05 did not submit any results for the characterisation campaign of the material.

6.4.1 Technical evaluation

The obtained data were first checked for compliance with the requested analysis protocol and for their validity based on technical reasons. The following criteria were considered during the evaluation:

- appropriate validation of the measurement procedure
- compliance with the analysis protocol: sample preparations and measurements performed on three days.
- absence of values given as below limit of quantification.

Based on the above criteria, no datasets were rejected, with the exception of L04 on the characterisation of PFPeA. Here five out of six individual values reported were declared as being below the method LOQ.

6.4.2 Statistical evaluation

The datasets accepted based on technical reasons were tested for normality of dataset means using kurtosis/skewness tests and normal probability plots and were tested for outlying means using the Grubbs test and using the Cochran test for outlying standard deviations, (both at a 99 % confidence level). Standard deviations within (s_{within}) and between ($s_{between}$) laboratories were calculated using one-way ANOVA. The results of these evaluations are shown in Table 7.

Table 7: Statistical evaluation of the technically accepted datasets for IRMM-428. *p*: number of technically valid datasets

Analyte in IRMM-428	*p*	Outliers		Normally distributed	Statistical parameters			
		Means	Variances		Mean [ng/L]	*s* [ng/L]	$s_{between}$ [ng/L]	s_{within} [ng/L]
L-PFOS	11	none	none	yes	9.636	1.332	1.271	0.974
PFOA	11	one	two	no	7.710	1.052	1.006	0.754
PFDA	11	one	none	no	4.451	1.450	1.432	0.546
PFBA	7	none	four	yes	10.663	6.903	6.943	1.045
PFPeA	7	none	one	yes	4.015	0.674	0.647	0.466
PFHxA	8	none	one	yes	7.383	0.830	0.799	0.548
PFHpA	8	none	none	yes	3.715	0.512	0.479	0.442
PFNA	8	none	none	yes	3.923	0.771	0.757	0.361
PFUnDA	7	none	one	yes	4.477	1.522	1.470	0.961
PFDoDA	6	none	one	n.d.[1)]	3.785	1.061	1.009	0.810
PFBS	8	none	two	yes	5.537	0.993	0.948	0.857
PFHxS	8	none	none	yes	3.616	0.437	0.422	0.279

[1)]not determined, p below 7

The laboratory means follow normal distributions for all PFASs with the exception of PFOA and PFDA. For L-PFOS, PFNA, PFHpA and PFHxS none of the data contains outlying means and variances. The datasets are therefore consistent and the mean of laboratory means is a good estimate of the true value. Standard deviations between laboratories are larger than the standard deviation within laboratories, showing that confidence intervals of replicate measurements are unsuitable as estimate of measurement uncertainty.

The statistical evaluation flags laboratory L01 as outlier for PFOA. As the difference between the mean value of laboratory L01 and the other results is not covered by the standard deviation and measurement uncertainty of laboratory L01 is not provided, there is evidence of a significant disagreement of results. As the technical evaluation of results did not indicate any technical flaws in any method, there is no reason for discarding any of the results. Since there is the possibility that the results of laboratory L01 are the only correct ones, no value is assigned for PFOA.

A similar situation occurs for PFDA, where laboratory L03 is identified as outlier. In this case the difference between the mean value of laboratory L03 and the other results is not covered by the measurement uncertainty of laboratory L03, therefore evidencing a significant disagreement of results. As the technical evaluation of results did not indicate any technical flaws in any method, there is no reason for discarding any of the results. Since there is the possibility that the results of laboratory L03 are the only correct ones, no value is assigned for measurand PFDA.

For PFBA two clusters of results are identified although not associated to any particular condition. In this case the disagreement of results and the lack of technical reason for the results grouping do not allow the assignment of a certified value to the compound.

The statistical evaluation flags a number of outlying variance for various PFASs including PFOA, PFBA, PFPeA, PFHxA, PFUnDA, PFDoDA and PFBS. This merely reflects the fact that different methods have different intrinsic variability. As all measurement methods were found technically sound, all results were retained.

It should be borne in mind that the methods used in the characterisation are methods routinely applied for measuring PFASs in water. The agreement of results from different methods demonstrates that the processing did not affect any properties relevant for these methods and that IRMM-428 behaves like a real sample.

The uncertainty related to the characterisation is estimated as the standard error of the mean of laboratory means (

Table 8).

Table 8: Uncertainty of characterisation for PFASs in IRMM-428

PFASs mass concentration IRMM-428	*p*	Mean [ng/L]	*s* [ng/L]	u_{char} [ng/L]
L-PFOS	11	9.636	1.332	0.402
PFPeA	7	4.015	0.674	0.254
PFHxA	8	7.383	0.830	0.293
PFHpA	8	3.715	0.512	0.181
PFNA	8	3.923	0.771	0.273
PFUnDA	7	4.477	1.522	0.575
PFDoDA	6	3.785	1.061	0.433
PFBS	8	5.537	0.993	0.351
PFHxS	8	3.616	0.437	0.155

7 Characterisation of calibrating solutions

Independent solutions of PFOA and PFDA, employed as common calibrants during the IRMM-428 characterisation campaign were purchased from Chiron AS, Trondheim. The solutions were prepared gravimetrically by dissolving amounts of neat crystalline PFOA and PFDA in methanol to obtain mass fractions of 2.5 µg/mL respectively.

Supplementary amounts of the neat crystalline PFOA and PFDA materials employed during preparation of the solutions were kindly provided by Chiron to IRMM for further assessment of their purity.

The purity analysis was performed by qNMR (Federal Institute for Materials Research and Testing (BAM), DE). Four replicate analyses per compound were carried out with an accuracy level of 0.5 %. Conditions of the analysis performed and results obtained are detailed in Annex E.

8 Value Assignment

Certified and indicative values were assigned.

Certified values are values that fulfil the highest standards of accuracy. Procedures at IRMM require generally pooling of not less than 6 datasets to assign certified values. Full uncertainty budgets in accordance with the 'Guide to the Expression of Uncertainty in Measurement' [4] were established.

Indicative values are values where either the uncertainty is deemed too large or where too few independent datasets were available to allow certification. Uncertainties are evaluated according to the same rules as for certified values.

8.1 Certified values and their uncertainties

The unweighted mean of the means of the accepted datasets as shown in Table was assigned as certified value for each parameter.

The assigned uncertainty consists of uncertainties related to characterisation, u_{char} (Section 6), potential between-unit inhomogeneity, u_{bb} (Section 4.1) and potential degradation during transport (u_{sts}) and long-term storage, u_{lts} (Section 5). For L-PFOS the uncertainty related to inhomogeneity u_{bb} was found to be negligible. These different contributions were combined to estimate the expanded, relative uncertainty of the certified value ($U_{CRM,\ rel}$) with a coverage factor k as:

$$U_{CRM,rel} = k \cdot \sqrt{u^2_{char,rel} + u^2_{bb,rel} + u^2_{sts,rel} + u^2_{lts,rel}} \qquad \textbf{Equation 8}$$

- u_{char} was estimated as described in Section 6
- u_{bb} was estimated as described in Section 4.1.
- u_{sts} was estimated as described in section 5.3
- u_{lts} was estimated as described in Section 5.3.

Because of the sufficient numbers of the degrees of freedom of the different uncertainty contributions, a coverage factor k of 2 was applied, to obtain the expanded uncertainties. The certified values and their uncertainties are summarised in Table 99.

Table 9: Certified values and their uncertainties for IRMM-428

PFASs mass fraction IRMM-428	Certified value [ng/L]	$u_{char, rel}$ [%]	$u_{bb, rel}$ [%]	$u_{sts, rel}$ [%]	$u_{lts, rel}$ [%]	$U_{CRM, rel}$ [%]	U_{CRM} [ng/L] [1)]
PFBS	5.5	6.34	4.84	2.51	9.10	24.7	1.4
PFHxS	3.6	4.27	4.37	2.69	11.00	25.7	1.0
L-PFOS	9.6	4.17	0.85[2)]	3.03	6.68	16.9	1.7
PFPeA	4.0	6.34	2.76	2.42	9.59	24.1	1.0
PFHxA	7.4	3.98	1.36	2.57	4.37	13.2	1.0
PFHpA	3.7	4.87	3.08	3.70	6.30	18.6	0.7

[1)]: Expanded (k = 2) and rounded uncertainty

[2)] Uncertainty was negligible compared to the main uncertainty contribution and not taken into account in Eq.8

8.2 Indicative values and their uncertainties

An indicative value was assigned for PFNA. Although the methodology applied for its determination was analogous to that employed for the determination of other PFASs, the total uncertainty associated to the assigned mass concentration value was considered too large. Indicative values may not be used as certified values. The uncertainty budgets were set up as for the certified values and are listed together with the assigned values in Table 10.

Table 10: Indicative values and their uncertainties for IRMM-428

PFASs mass fraction IRMM-428	Indicative value [ng/L]	$u_{char, rel}$ [%]	$u_{bb, rel}$ [%]	$u_{sts, rel}$ [%]	$u_{lts, rel}$ [%]	$U_{CRM, rel}$ [%]	U_{CRM} [ng/L] [1)]
PFNA	3.9	6.95	9.64	3.24	11.73	34.0	1.4

[1)]: Expanded (k = 2) and rounded uncertainty

9 Metrological traceability and commutability

9.1 Metrological traceability

Identity

PFASs are chemically clearly defined analytes. Identity was confirmed by mass spectrometry. The participants used different methods for the sample preparation as well as for the final determination, demonstrating absence of measurement bias. The measurands are therefore structurally defined and independent of the measurement method.

Quantity value

Only validated methods were used for the determination of the assigned values. Different calibrants of known purity and specified traceability of their assigned values were used and all relevant input parameters were calibrated. The individual results are therefore traceable to the SI, as it is also confirmed by the agreement among the technically accepted datasets. As the assigned values are combinations of agreeing results individually traceable to the SI, the assigned quantity values themselves are traceable to the SI as well.

9.2 Commutability

Many measurement procedures include one or more steps, which are selecting specific (or specific groups of) analytes from the sample for the subsequent steps of the whole measurement process. Often the complete identity of these 'intermediate analytes' is not fully known or taken into account. Therefore, it is difficult to mimic all the analytically relevant properties of real samples within a CRM. The degree of equivalence in the analytical behaviour of real samples and a CRM with respect to various measurement procedures (methods) is summarised in a concept called 'commutability of a reference material'. There are various definitions expressing this concept. For instance, the CSLI Guideline C-53A [18] recommends the use of the following definition for the term *commutability*:

> "The equivalence of the mathematical relationships among the results of different measurement procedures for an RM and for representative samples of the type intended to be measured."

The commutability of a CRM defines its fitness for use and, thus, is a crucial characteristic in case of the application of different measurement methods. When commutability of a CRM is not established in such cases, the results from routinely used methods cannot be legitimately compared with the certified value to determine whether a bias does not exist in calibration, nor can the CRM be used as a calibrant.

IRMM-428 was produced from drinking water spiked with a mixture of PFASs. The analytical behaviour will be the same as for a routine sample of drinking water.

10 Instructions for use

10.1 Safety information

For laboratory use only. The usual laboratory safety measures apply.

10.2 Storage conditions

The materials shall be stored at 4 °C ± 3 °C in the dark. The user is reminded to close bottles tightly immediately after taking a sample to avoid evaporation of water.

Please note that the European Commission cannot be held responsible for changes that happen during storage of the material at the customer's premises, especially of opened bottles.

10.3 Preparation and use of the material

To make it ready for use, the material has to be handled according to the following procedure prior to analysis:

- Re-homogenize the liquid samples by ultrasonication for 10 minutes followed by 1 hour of shaking at room temperature[5]
- Sample intake (a minimum aliquot of 100 mL) should be performed immediately after shaking
- The addition of an internal standard to the sample is done immediately after sample intake.

[5] The thermal expansion of the water at room temperature in an analytical laboratory (22 ºC ± 3 ºC) is covered by the uncertainty of the certified values.

The use of PTFE or other fluoropolymers during sample extraction and analyses must be avoided [19]. In case the analytical system employed for the PFASs determination does contain PTFE or other fluoropolymers, the PFASs leaching from these polymers may be retained by an additional column installed just prior to the injection valve. In addition, the replacement of fluoropolymer tubing by non-fluorinated polymer tubing or stainless steel reduces leaching of PFASs and therefore reduces potential contamination of the sample extract.

10.4 Minimum sample intake

The minimum sample intake representative for all parameters is 100 mL.

10.5 Use of the certified value

The main purpose of these materials is to assess method performance, i.e. for checking accuracy of analytical results/calibration. As any reference material, it can also be used for control charts or validation studies.

Use as a calibrant

It is not recommended to use this matrix material as calibrant. If used nevertheless, the uncertainty of the certified value shall be taken into account in the estimation of the measurement uncertainty.

Comparing an analytical result with the certified value

A result is unbiased if the combined standard uncertainty of measurement and certified value covers the difference between the certified value and the measurement result (see also ERM Application Note 1, www.erm-crm.org [20].

For assessing the method performance, the measured values of the CRMs are compared with the certified values. The procedure is described here in brief:

- Calculate the absolute difference between mean measured value and the certified value (Δ_{meas}).
- Combine measurement uncertainty (u_{meas}) with the uncertainty of the certified value (u_{CRM}): $u_{\Delta} = \sqrt{u_{meas}^2 + u_{CRM}^2}$
- Calculate the expanded uncertainty (U_{Δ}) from the combined uncertainty ($u_{\Delta,}$) using an appropriate coverage factor, corresponding to a level of confidence of approximately 95 %
- If $\Delta_{meas} \leq U_{\Delta}$ no significant difference between the measurement result and the certified value, at a confidence level of about 95 % exists.

Use in quality control charts

The materials can be used for quality control charts. Different CRM-units will give the same result as inhomogeneity was included in the uncertainties of the certified values.

11 Acknowledgments

The authors would like to acknowledge the support received from C. Contreras concerning the set-up of the required isochronous studies.

Chiron AS is acknowledged for the complementary provision of neat crystalline material of PFOA and PFDA for purity assessment purposes.

Wellington Laboratories Inc. is acknowledged for the complementary provision of spiking solutions employed during the processing of the CRM.

Laboratories associated to the PERFOOD consortium are acknowledged for their participation in the IRMM-428 material certification project.

3M Laboratory is acknowledged for their complimentary participation in the characterisation campaign of IRMM-428.

Furthermore, the authors would like to thank L. Deprez and C. Venchiarutti (IRMM) for the reviewing of the certification report, as well as the experts of the Certification Advisory Panel "small organic molecules", S. De Saeger (Ghent University, BE), Alexey Solyakov (National Veterinary Institute, SE) and S. van Leeuwen (RIKILT, Wageningen University and Research Centers, NL) for their constructive comments.

12 References

1 ISO Guide 34, General requirements for the competence of reference materials producers, International Organization for Standardization, Geneva, Switzerland, 2009

2 ISO Guide 35, Reference materials – General and statistical principles for certification, International Organization for Standardization, Geneva, Switzerland, 2006

3 ISO/IEC 17025, General Requirements for the Competence of Testing and Calibration Laboratories; International Organization for Standardization, Geneva, Switzerland, 2005

4 ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement, (GUM 1995), International Organization for Standardization, Geneva, Switzerland, 2008

5 R.C. Buck, J. Franklin, U. Berger, J.M. Conder, I.T. Cousins, P. de Voogt, A.A. Jensen, K. Kannan, S.A. Mabury and S.P.J. van Leeuwen, Perfluoroalkyl and Polyfluoroalkyl Substances in the Environment: Terminology, Classification, and Origins. Integrated Environmental Assessment and Management, 2011. **7**(4): p. 513–541.

6 Perfluoroalkylated substances in food: occurrence and dietary exposure. EFSA Journal 2012, 2012(10(6)).

7 COMMISSION REGULATION (EU) No 756/2010 of 24 August 2010 amending Regulation (EC) No 850/2004 of the European Parliament and of the Council on persistent organic pollutants as regards Annexes IV and V in Official Journal of the European Union. 2010.

8 COMMISSION REGULATION (EU) No 757/2010 of 24 August 2010 amending Regulation (EC) No 850/2004 of the European Parliament and of the Council on persistent organic pollutants as regards Annexes I and III in COMMISSION REGULATION (EU) No 757/2010. 2010, Official Journal of the European Union: Official Journal of the European Union.

9 Draft toxicological profile for perfluoralkyls. U.S. Department of Health and Human Services, Public Health Service Agency for Toxic Substances and Disease Registry (ATSDR), 2009.

10 Proposal for a directive fo the European Parliament and of the council amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy, COM(2001) 876, European Commission, Editor. 2011.

11 Opinion of the Scientific panel on contaminants in the food chain on perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts. The EFSA Journal 2008, p. 1-131.

12 J.M. Weiss I. van der Veen, J. de Boer, S.P.J. van Leeuwen, W. Cofino, S. Crum. Analytical improvements shown over four interlaboratory studies of perfluoroalkyl substances in environmental and food samples. TrAC Trends in Analytical Chemistry, 2013. 43(0): p. 204-216.

13 S.P.J. van Leeuwen, C.P. Swart, I. van der Veen, J. de Boer, Significant improvements in the analysis of perfluorinated compounds in water and fish: Results from an interlaboratory method evaluation study. Journal of Chromatography A, 2009. 1216(3) 401-409.

14 G. Lindstrom, A. Karrman, B. van Bavel, Accuracy and precision in the determination of perfluorinated chemicals in human blood verified by interlaboratory comparison. Journal of Chromatography A, 2009. 1216(3): p. 394-400

15 T.P.J. Linsinger, J. Pauwels, A.M.H. van der Veen, H. Schimmel, A. Lamberty, Homogeneity and stability of reference materials, Accred. Qual. Assur. 6 (2001) 20-25

16 A. Lamberty, H. Schimmel, J. Pauwels, The study of the stability of reference materials by isochronous measurements, Fres. J. Anal. Chem. 360 (1998) 359-361

17 T.P.J. Linsinger, J. Pauwels, A. Lamberty, H. Schimmel, A.M.H. van der Veen, L. Siekmann, Estimating the Uncertainty of Stability for Matrix CRMs, Fres. J. Anal. Chem. 370 (2001) 183-188

18 H. Vesper, H. Emons, M. Gnezda, C. P. Jain, W. G. Miller, R. Rej, G. Schumann, J. Tate, L. Thienpont, J. E. Vaks, Characterization and Qualification of Commutable Reference Materials for Laboratory Medicine; Approved Guideline, CLSI document C53-A, Clinical and Laboratory Standards Institute, Wayne, PA, USA, 2010

19 A. Ballesteros-Gomez, S. Rubio, S. van Leeuwen, Tetrahydrofuran-water extraction, in-line clean-up and selective liquid chromatography/tandem mass spectrometry for the quantitation of perfluorinated compounds in food at the low picogram per gram level. J. Chromatogr A, 2010. 1217(24) 5913-5921

20 T.P.J. Linsinger, ERM Application Note 1: Comparison of a measurement result with the certified value, www.erm-crm.org (last accessed on 11/10/2014)

CRM Water: Perfood

Solution/Mixture of Native Perfluoroalkylcarboxylic Acids and Native Perfluoroalkylsulfonates

PRODUCT CODE:	CRM Water: Perfood
LOT NUMBER:	111011
SOLVENT(S):	Methanol / Water (<1%)
DATE PREPARED: (mm/dd/yyyy)	11/11/2011
LAST TESTED: (mm/dd/yyyy)	11/15/2011
EXPIRY DATE: (mm/dd/yyyy)	11/15/2014
RECOMMENDED STORAGE:	Store ampoule in a cool, dark place

DESCRIPTION:

CRM Water: Perfood is a solution/mixture of nine native perfluoroalkylcarboxylic acids (C_4-C_{12}) and five native perfluoroalkylsulfonates (C_4, C_6-C_8, and C_{10}). The full name, abbreviation and concentration for each of the components are given in Table A.

The individual perfluoroalkylcarboxylic acids and perfluoroalkylsulfonates all have chemical purities of >98%.

DOCUMENTATION/ DATA ATTACHED:

Table A: Components and Concentrations of the Solution/Mixture
Figure 1: LC/MS Data (SIR)
Figure 2: LC/MS/MS Data of Stock Solution (Selected MRM Transitions)
Figure 3: LC/MS/MS Data of Stock Solution (Selected MRM Transitions)

ADDITIONAL INFORMATION:

- See page 2 for further details.
- Contains 4 mole eq. of NaOH to prevent conversion of the carboxylic acids to their respective methyl esters.

FOR LABORATORY USE ONLY: NOT FOR HUMAN OR DRUG USE

Wellington Laboratories Inc., 345 Southgate Dr. Guelph ON N1G 3M5 CANADA
519-822-2436 • Fax: 519-822-2849 • info@well-labs.com

INTENDED USE:

The products prepared by Wellington Laboratories Inc. are for laboratory use only. They are designed to be used as reference standards for the identification and/or quantification of specific chemical compound(s).

HAZARDS:

This product should only be used by qualified personnel familiar with its potential hazards and trained in the handling of hazardous chemicals. Due care should be exercised to prevent unnecessary human contact or ingestion. All procedures should be carried out in a well-functioning fume hood and suitable gloves, eye protection and clothing should be worn at all times. Waste should be disposed of according to national and regional regulations. Material Safety Data Sheets (MSDSs) are available upon request.

SYNTHESIS / CHARACTERIZATION:

Where possible, all of our products are synthesized using single-product, unambiguous routes. They are then characterized, and their structures and purities confirmed, using a combination of the most relevant techniques, such as NMR, GC/MS, LC/MS/MS, x-ray crystallography and melting point. Isotopic purities of mass-labelled compounds are also confirmed using HRGC/HRMS and/or LC/MS/MS.

HOMOGENEITY:

Prior to solution preparation, crystalline material is tested for homogeneity using a variety of techniques (as stated above) and its solubility in a given diluent is taken into consideration. Duplicate solutions of a new product are prepared from the same crystalline lot and, after the addition of an appropriate internal standard, they are compared by GC/MS and/or LC/MS/MS. The relative response factors of the analyte of interest in each solution are required to be <5% RSD. New solution lots of existing products are compared to older lots in the same manner, which further confirms the homogeneity of the crystalline material as well as the stability and homogeneity of the solutions in the storage containers.

UNCERTAINTY:

The maximum combined relative standard uncertainty of our reference standard solutions is calculated using the following equation:

The combined relative standard uncertainty, $u_c(y)$, of a value y and the uncertainty of the independent parameters x_1, x_2,...x_n on which it depends is:

$$u_c(y(x_1, x_2, ...x_n)) = \sqrt{\sum_{i=1}^{n} u(y, x_i)^2}$$

where x is expressed as a relative standard uncertainty of the individual parameter.

The individual uncertainties taken into account include those associated with weights (calibration of the balance) and volumes (calibration of the volumetric glassware). An expanded maximum combined percent relative uncertainty of ±5% (calculated with a coverage factor of 2 and a level of confidence of 95%) is stated on the Certificate of Analysis for all our products.

TRACEABILITY:

All reference standard solutions are traceable to specific crystalline lots. The microbalances used for solution preparation are regularly tested by an external, ISO/IEC 17025:2005 accredited calibration company. In addition, their calibration is verified prior to each weighing using NIST and/or NRC traceable external weights. All volumetric glassware used is of Class A tolerance and has been tested according to the appropriate ASTM procedures, which are ultimately traceable to NIST. For certain products, traceability to international interlaboratory studies has also been established.

EXPIRY DATE / PERIOD OF VALIDITY:

Ongoing stability studies of this product have demonstrated stability in its composition and concentration for the period of time specified by the expiry date in the unopened ampoule. Monitoring for any degradation or change in concentration of the listed analyte(s) is performed on a routine basis.

LIMITED WARRANTY:

At the time of shipment, all products are warranted to be free of defects in material and workmanship and to conform to the stated technical and purity specifications.

For additional information or assistance concerning this or any other products from Wellington Laboratories Inc., please visit our website at www.well-labs.com or contact us directly at info@well-labs.com

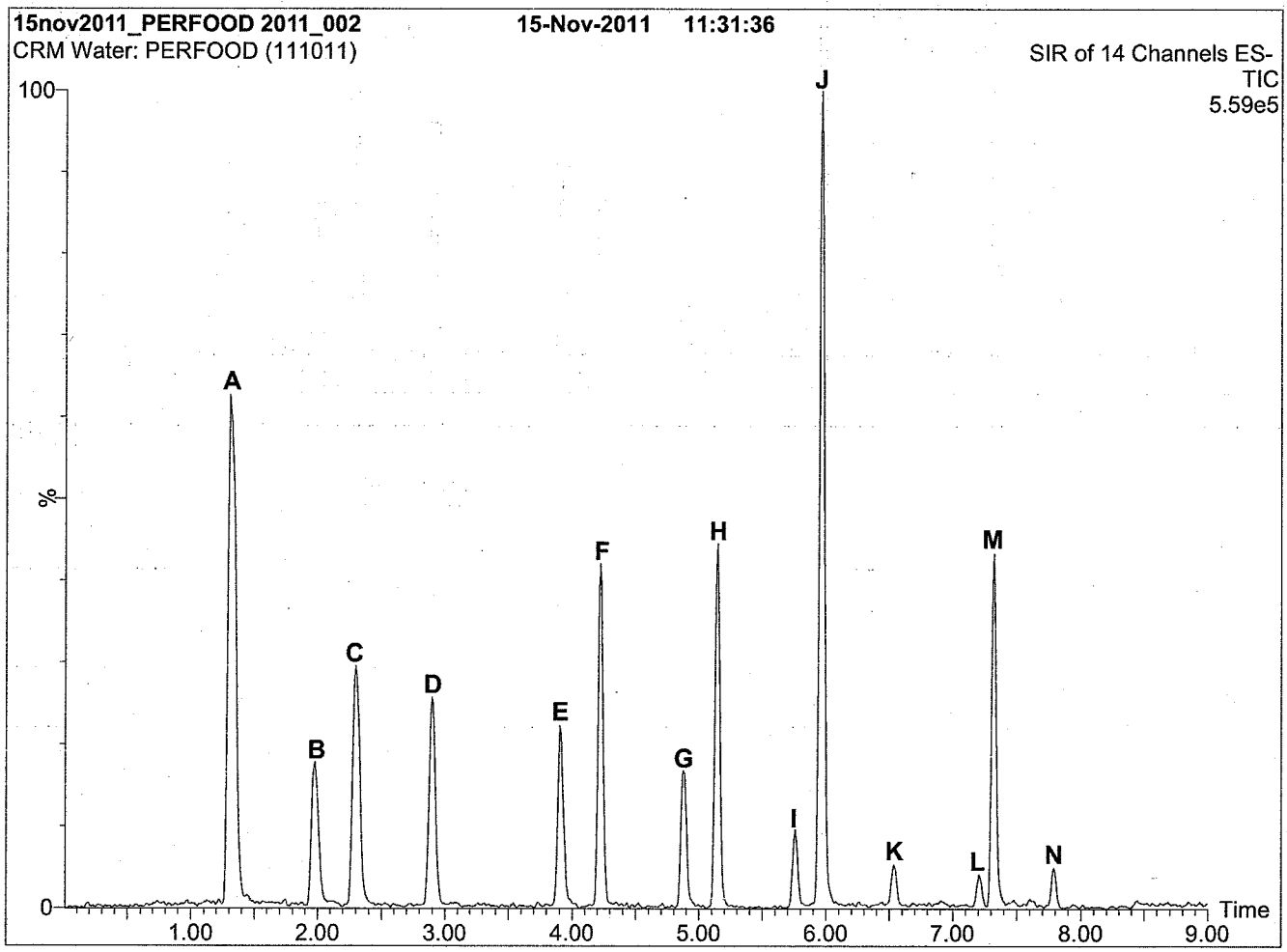
Table A: **CRM Water: Perfood; Components and Concentrations (µg/ml, ± 5% in Methanol / Water (<1%))**

Name	Abbreviation	Concentration (µg/ml)	Peak Assignment in Figure 1
Perfluoro-n-butanoic acid	PFBA	0.40	A
Perfluoro-n-pentanoic acid	PFPeA	0.25	B
Perfluoro-n-hexanoic acid	PFHxA	0.50	D
Perfluoro-n-heptanoic acid	PFHpA	0.25	E
Perfluoro-n-octanoic acid	PFOA	0.50	G
Perfluoro-n-nonanoic acid	PFNA	0.25	I
Perfluoro-n-decanoic acid	PFDA	0.25	K
Perfluoro-n-undecanoic acid	PFUdA	0.25	L
Perfluoro-n-dodecanoic acid	PFDoA	0.25	N

Name	Abbreviation	Concentration (µg/ml)		Peak Assignment in Figure 1
		as the salt	as the anion	
Potassium perfluoro-1-butanesulfonate	L-PFBS	0.40	0.35	C
Sodium perfluoro-1-hexanesulfonate	L-PFHxS	0.25	0.24	F
Sodium perfluoro-1-heptanesulfonate	L-PFHpS	0.25	0.24	H
Sodium perfluoro-1-octanesulfonate	L-PFOS	0.65	0.62	J
Sodium perfluoro-1-decanesulfonate	L-PFDS	0.25	0.24	M

Certified By: B.G. Chittim **Date:** 11/17/2011 (mm/dd/yyyy)

Figure 1: CRM Water: Perfood; LC/MS Data (Total Ion Current Chromatogram; SIR)



Conditions for Figure 1:

LC: **Waters Acquity Ultra Performance LC**
MS: **Micromass Quattro *micro* API MS**

Chromatographic Conditions

Column: Acquity UPLC BEH Shield RP_{18}
1.7 µm, 2.1 x 100 mm

Mobile phase: Gradient
Start: 60% H_2O / 40% (80:20 MeOH:ACN)
(both with 10 mM NH_4OAc buffer)
Ramp to 90% organic over 9 min, hold for 1.5 min before returning to initial conditions in 0.5 min.

Time: 12 min

Flow: 300 µl/min

MS Parameters

Experiment: SIR of 14 Channels

Source: Electrospray (negative)
Capillary Voltage (kV) = 2.50
Cone Voltage (V) = variable (10-70)
Cone Gas Flow (l/hr) = 100
Desolvation Gas Flow (l/hr) = 750

Figure 2: **CRM Water: Perfood; LC/MS/MS Data (Selected MRM Transitions)**

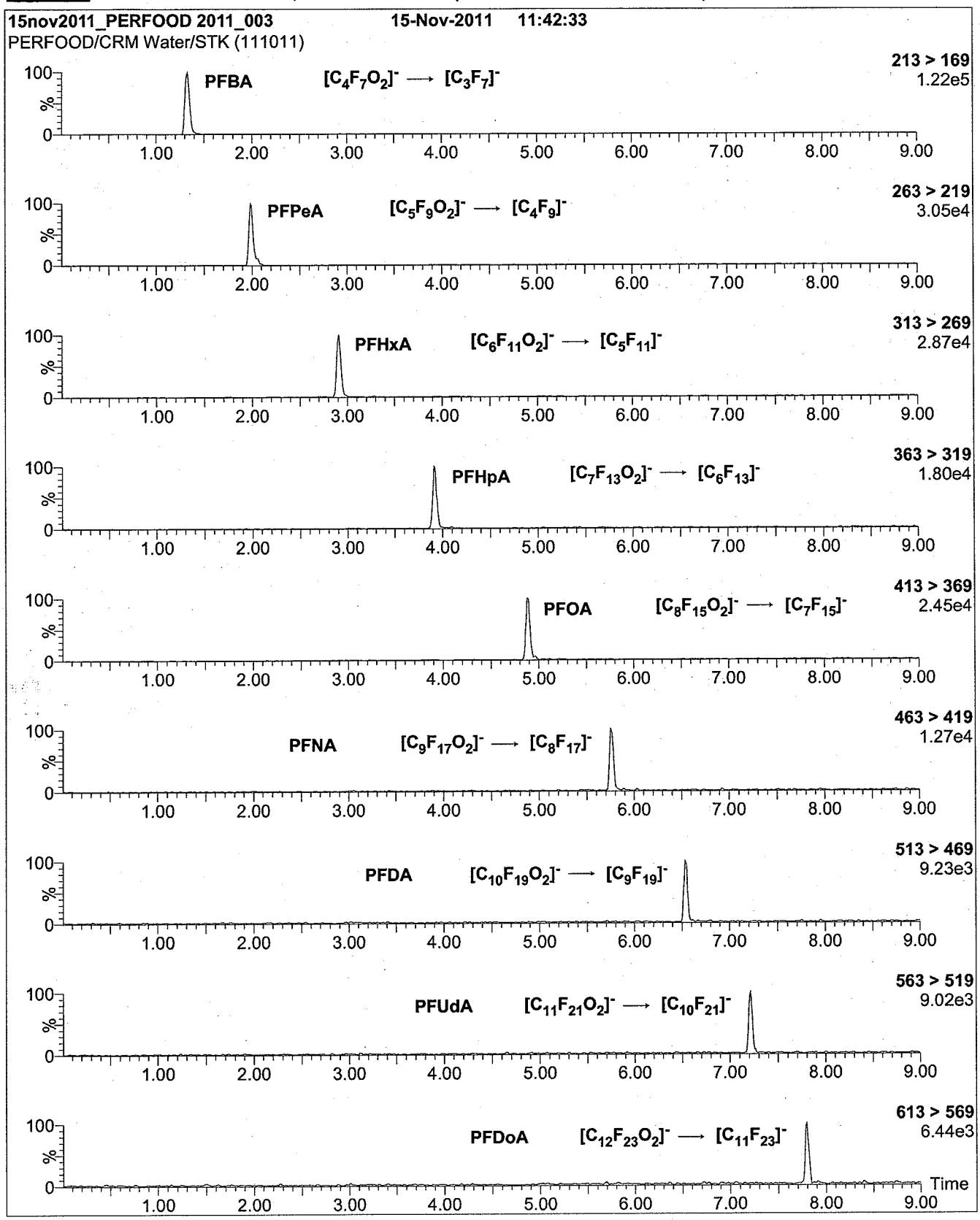
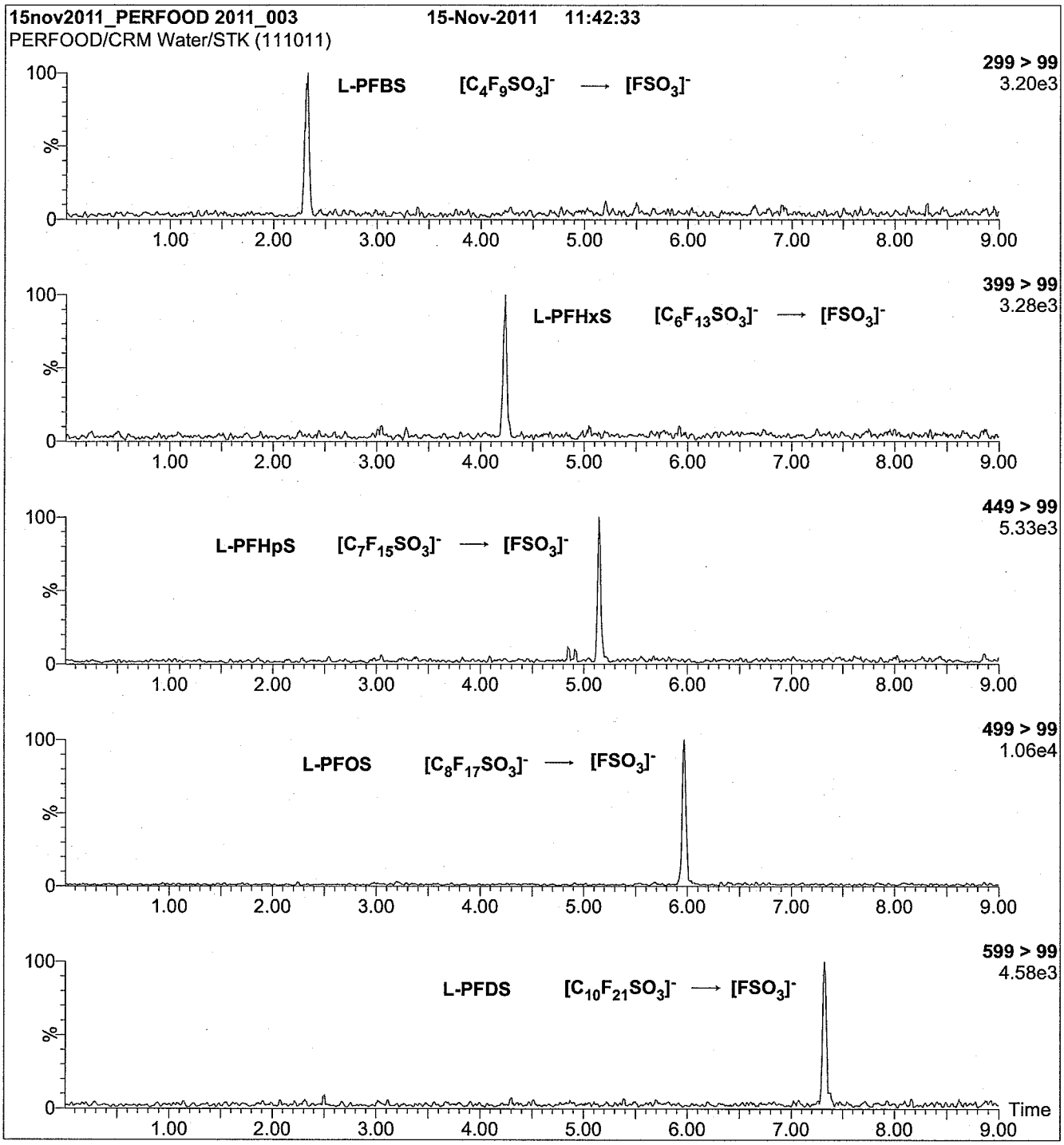


Figure 3: CRM Water: Perfood; LC/MS/MS Data (Selected MRM Transitions)



Conditions for Figures 2 and 3:

Injection: on-column (CRM Water: Perfood)

Mobile phase: Same as Figure 1

Flow: 300 µl/min

MS Parameters
Collision Gas (mbar) = 3.35e-3
Collision Energy (eV) = 8-50 (variable)

WELLINGTON LABORATORIES

345 Southgate Drive
Guelph ON N1G 3M5 CANADA
Tel: 519-822-2436
Fax: 519-822-2849

PACKING SLIP

ORDER NUMBER: PERFOOD project
DATE OF ORDER: November 15/2011
DATE SHIPPED: November 17/2011

CLIENT:
VU University of Amsterdam
Institute for Environmental Studies (IVM)
Faculty of Earth and Life Sciences (FALW)
De Boelelaan 1085
1081 HV Amsterdam
THE NETHERLANDS
ATTENTION: Ms. Ike van der Veen

DETAILS OF ORDER

Chemical	Unit Quantity	Total Units
CRM Water	1.2 ml	20
M5PFPeA	1.2 ml	10
M5PFHxA	1.2 ml	10
M4PFHpA	1.2 ml	10

ANNEX B. Analytical results obtained by LC-MS/MS for the homogeneity study of PFASs (ng/L) in IRMM-428 material. Triplicate analysis per bottle was performed. Outliers detected for individual results are highlighted in italic.

IRMM-428 Bottle No	PFOA	PFDA	L-PFOS	PFBA	PFPeA	PFHxA	PFHpA	PFNA	PFBS	PFHxS
73	7.80	2.73	7.73	6.30	5.90	7.33	3.67	3.27	5.80	3.53
73	7.60	2.23	7.03	6.10	5.47	7.27	3.40	2.70	5.63	3.20
73	7.47	2.10	7.57	6.23	5.47	7.10	3.77	2.80	5.37	3.30
145	7.63	2.40	7.57	6.23	5.30	7.20	3.27	3.13	5.60	3.43
145	7.47	2.37	7.50	6.17	5.50	6.87	3.27	2.97	5.10	3.27
145	7.60	2.30	7.23	6.43	5.40	6.97	3.33	2.97	5.73	3.30
270	7.60	2.67	7.33	6.23	5.57	7.57	3.83	3.00	5.80	3.27
270	7.57	2.67	7.53	5.93	5.90	7.17	3.80	3.13	5.57	3.37
270	7.47	2.13	7.27	5.97	5.37	7.20	3.67	2.90	5.43	3.30
329	7.60	2.50	7.27	6.03	5.63	7.40	3.50	2.60	5.57	3.50
329	7.67	2.63	7.53	6.17	5.47	7.53	3.87	3.00	5.47	3.23
329	7.50	2.57	7.20	5.77	5.50	7.30	3.47	2.90	5.33	3.30
456	7.63	2.67	7.47	6.00	5.53	7.43	3.90	3.47	5.10	3.37
456	7.43	2.80	7.43	6.33	5.70	7.50	3.93	3.07	5.53	3.27
456	7.43	2.47	7.43	5.77	5.30	7.30	3.63	2.70	5.40	3.07
543	7.40	2.77	7.13	6.07	5.13	7.13	3.73	2.83	5.03	3.37
543	7.40	2.60	7.33	5.93	5.10	7.27	3.57	3.07	5.23	3.10
543	7.63	2.27	7.57	5.77	5.23	7.10	3.80	2.90	5.30	3.33
625	7.43	2.70	7.43	5.57	5.27	6.93	3.83	2.77	5.40	3.10
625	7.37	2.70	7.53	6.13	5.37	7.33	3.90	2.93	5.23	3.30
625	7.47	2.70	7.40	6.00	5.47	7.47	4.03	2.90	5.33	3.20
698	7.47	2.37	7.37	6.30	5.57	6.87	3.83	2.63	5.47	3.17
698	7.57	2.73	7.53	5.77	5.47	7.33	3.70	3.03	5.63	3.73
698	7.27	2.63	7.33	5.93	4.87	7.53	3.73	2.87	5.23	3.10
804	7.36	2.47	7.27	6.19	5.60	7.50	3.30	2.71	4.95	3.20
804	7.38	2.53	7.13	6.32	5.61	7.35	3.39	2.59	5.11	3.20
804	7.43	2.80	7.80	6.10	5.68	7.15	3.99	2.93	5.31	3.17
891	7.40	2.60	7.43	6.60	5.81	7.11	3.31	2.90	4.96	3.27
891	7.45	2.47	7.53	6.20	5.56	7.00	3.77	2.74	5.27	3.23

891	7.34	3.07	7.87	6.33	*6.24*	7.41	3.58	2.53	4.92	3.10
988	7.28	2.47	7.47	6.18	5.39	6.97	3.58	2.81	4.73	2.93
988	7.43	2.50	7.50	6.18	5.57	6.86	3.54	2.88	4.86	2.87
988	7.45	2.47	7.63	6.20	5.64	7.42	3.44	2.91	5.22	3.20
1073	7.39	2.63	7.60	5.87	5.26	6.74	3.80	2.84	4.57	2.93
1073	7.12	2.10	7.13	6.27	5.64	7.02	3.47	2.60	4.88	2.67
1073	7.50	2.53	7.57	5.94	5.58	6.85	3.52	2.99	4.70	2.97
1182	7.52	2.63	7.43	6.37	4.75	7.29	3.32	**1.59*	4.98	3.13
1182	7.38	1.97	7.20	6.29	5.29	7.11	3.60	2.81	5.23	3.10
1182	7.67	1.97	6.87	6.18	5.42	6.89	3.85	2.69	5.07	3.27
1283	7.50	2.10	7.13	6.10	5.03	7.10	3.47	2.73	5.33	3.10
1283	7.43	2.57	7.10	6.23	5.40	7.33	3.47	2.53	5.37	3.50
1283	7.43	2.33	7.30	6.37	5.43	7.53	3.70	2.83	5.53	3.17

* Mean value for PFNA in bottle 1182 is identified as outlier

ANNEX C. Analytical results obtained from the short-term stability study of PFASs in IRMM-428. Three replicates per IRMM-428 bottle were carried out and results are expressed as ng/L for each individual PFASs compound. Outlier results are highlighted in italic.

StorageT [ºC]	Storage t [weeks]	IRMM-428 bottle n.	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	L-PFOS	PFDA	PFBS	PFHxS
-70	0	672	6.15	3.61	6.43	3.61	5.84	3.36	8.67	3.79	4.89	3.39
-70	0	672	6.12	3.48	6.25	3.85	5.78	3.03	7.59	3.22	5.05	3.31
-70	0	672	6.76	3.51	6.53	3.68	5.33	3.43	6.69	2.79	4.78	3.37
-70	0	18	6.07	3.58	6.02	4.25	5.04	2.76	9.86	3.29	4.79	2.79
-70	0	18	7.36	3.78	6.60	4.08	5.06	2.38	5.94	2.53	4.95	3.02
-70	0	18	6.78	3.42	6.96	*5.20*	*8.32*	*4.92*	*11.58*	4.48	5.01	*4.51*
-20	1	48	7.05	3.91	6.69	4.13	5.98	3.55	9.11	3.88	4.95	3.28
-20	1	48	6.90	4.00	6.58	3.94	5.67	3.03	7.02	2.94	4.97	3.16
-20	1	48	6.20	3.09	5.80	3.66	5.71	3.09	7.02	2.85	4.25	3.40
-20	1	679	5.74	2.95	4.23	2.17	2.75	2.03	6.01	2.72	3.44	1.77
-20	1	679	6.86	4.13	8.10	4.71	7.46	3.70	7.98	3.21	6.24	4.20
-20	1	679	6.13	3.59	6.90	4.40	7.12	4.71	10.59	4.22	5.32	3.95
-20	2	714	4.79	2.65	4.51	2.60	4.22	2.73	6.64	3.19	3.54	2.50
-20	2	714	6.27	3.66	6.76	3.94	6.65	3.27	7.69	3.32	5.13	3.52
-20	2	714	7.70	4.39	8.39	4.71	6.63	3.90	8.74	3.51	6.30	3.98
-20	2	58	5.99	3.62	6.45	3.93	5.58	3.41	8.03	3.59	4.93	3.28
-20	2	58	6.23	3.52	6.73	3.49	6.25	3.11	7.38	2.82	5.06	3.47
-20	2	58[1]										
-20	4	91	5.77	3.24	6.31	3.26	6.27	3.69	9.74	4.19	4.64	3.50
-20	4	91	6.29	3.76	6.70	4.10	5.42	2.75	7.48	2.72	5.14	3.61
-20	4	91	5.99	3.37	6.35	3.97	5.93	2.75	6.85	2.87	5.05	3.46
-20	4	735	4.11	2.13	3.64	2.47	3.66	2.38	6.26	2.72	2.82	2.24
-20	4	735	6.38	3.63	6.84	3.89	5.94	3.03	6.29	2.80	5.26	3.29
-20	4	735	9.33	5.53	9.57	4.88	7.99	4.97	11.98	5.05	7.31	4.60
4	1	110	6.34	3.51	6.33	3.74	6.47	3.31	8.23	3.87	4.89	3.23
4	1	110	5.85	3.47	6.24	3.80	5.91	3.13	7.80	3.34	5.04	3.23

[1] Technical problems with the sample. No results reported.

StorageT [ºC]	Storage t [weeks]	IRMM-428 bottle n.	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	L-PFOS	PFDA	PFBS	PFHxS
4	1	110	6.52	3.65	6.51	3.96	5.36	3.15	7.52	2.94	4.99	3.46
4	1	765	3.88	1.92	3.75	2.60	4.25	3.23	8.56	4.21	2.83	2.43
4	1	765	4.24	2.30	4.25	2.87	4.41	2.86	5.61	2.57	3.11	2.59
4	1	765	*12.75*	*6.06*	*13.40*	*6.37*	*10.09*	*5.08*	10.11	4.42	*10.51*	*5.30*
4	2	776	5.80	3.43	6.19	3.65	5.75	3.38	8.25	3.62	5.06	3.25
4	2	776	5.87	3.70	6.29	3.69	5.72	3.39	7.58	3.28	4.76	3.19
4	2	776	5.82	2.87	6.45	3.05	5.84	3.29	6.73	3.08	4.75	3.23
4	2	128	5.37	3.39	6.39	3.63	5.73	3.26	8.49	3.85	5.06	3.13
4	2	128	5.58	3.38	6.20	3.75	5.56	3.00	7.95	3.29	4.95	3.24
4	2	128	5.81	3.62	6.29	3.77	5.59	2.81	7.80	3.26	5.37	3.27
4	4	174	5.67	3.49	6.17	3.77	5.85	3.40	8.13	3.60	5.29	3.43
4	4	174	6.85	3.58	6.45	3.58	5.72	3.25	7.40	3.17	4.97	3.31
4	4	174	7.81	3.58	6.36	3.82	5.46	3.29	7.29	2.99	5.01	3.21
4	4	806	5.64	3.41	6.35	3.64	5.72	3.63	8.48	3.73	4.90	3.24
4	4	806	5.77	3.59	6.42	3.61	5.66	3.14	7.51	3.38	4.92	3.21
4	4	806	5.97	3.61	6.55	3.75	5.25	3.11	7.22	3.07	4.86	3.24
18	1	845	5.70	3.74	6.43	3.70	5.93	3.27	7.36	3.41	4.79	3.28
18	1	845	6.14	3.71	6.48	3.83	5.68	3.13	7.05	3.17	4.71	3.03
18	1	845	5.93	3.67	6.45	3.63	5.44	3.44	7.09	2.96	4.73	3.19
18	1	185	6.30	3.68	6.03	3.82	5.96	3.23	8.12	3.54	4.73	3.35
18	1	185	7.34	4.21	7.03	4.30	6.14	3.35	8.00	3.44	5.55	3.43
18	1	185	7.70	3.73	5.97	3.73	5.20	3.13	8.37	3.46	4.90	3.29
18	2	209	7.05	3.87	6.81	3.96	6.45	3.65	8.69	3.92	5.20	3.39
18	2	209	7.18	3.71	6.90	3.87	6.03	3.28	7.38	3.08	5.14	3.48
18	2	209	5.57	3.14	5.20	3.35	4.81	3.56	8.24	3.37	4.17	2.94
18	2	871	4.14	2.63	4.98	2.90	4.69	2.95	6.20	2.99	3.52	2.60
18	2	871	6.77	3.77	7.30	3.44	6.66	3.33	8.13	3.52	5.31	3.36
18	2	871	7.07	4.32	7.31	4.24	5.87	3.30	7.53	3.39	5.42	3.51
18	4	248	6.21	3.66	6.64	3.74	6.02	3.41	8.73	3.46	5.01	3.17
18	4	248	6.04	3.72	6.45	3.73	5.53	3.06	7.33	3.09	5.03	3.26
18	4	248	6.51	3.67	6.37	3.82	5.96	3.49	8.63	3.65	4.83	3.37
18	4	895	4.53	2.86	5.27	3.20	5.15	3.10	7.29	3.16	3.88	2.79

StorageT [ºC]	Storage t [weeks]	IRMM-428 bottle n.	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	L-PFOS	PFDA	PFBS	PFHxS
18	4	895	6.30	3.76	7.08	3.82	6.13	3.60	8.03	3.66	5.51	3.47
18	4	895	6.59	4.01	7.02	3.99	5.20	2.86	6.52	2.73	5.48	3.40
60	1	905	3.54	1.82	4.01	3.09	3.30	1.87	4.90	2.57	3.04	1.96
60	1	905	6.92	3.39	8.16	6.07	7.00	3.74	8.86	3.65	6.19	3.91
60	1	905	7.03	3.35	8.08	5.96	6.63	4.16	8.76	3.69	6.84	3.71
60	1	272	6.04	2.71	6.53	5.40	5.48	3.39	8.16	3.59	4.89	3.21
60	1	272	6.29	2.81	6.76	5.19	5.56	2.95	7.68	3.23	5.19	3.06
60	1	272	7.67	2.71	7.15	6.08	5.75	3.42	8.38	3.48	5.71	3.85
60	2	281	6.82	2.72	6.88	5.80	5.97	3.05	8.13	3.71	5.47	3.20
60	2	281	6.51	2.64	6.60	5.48	5.85	3.32	8.13	3.48	5.37	3.29
60	2	281	6.87	2.63	6.63	5.56	5.47	3.58	7.48	3.25	5.19	3.37
60	2	932	4.18	1.92	4.92	3.86	4.64	2.91	7.06	3.31	3.89	2.70
60	2	932	5.14	2.37	5.74	3.73	3.86	2.08	4.76	2.18	4.83	2.51
60	2	932	8.90	4.08	*10.50*	8.26	9.42	5.07	11.45	4.56	7.75	*5.31*
60	4	975	5.99	2.32	6.41	5.18	5.69	3.44	7.32	2.96	5.61	3.09
60	4	975	5.93	2.39	6.68	4.84	5.68	3.22	7.69	3.33	5.86	3.27
60	4	975	5.90	2.60	6.47	4.98	5.13	3.11	7.78	3.14	5.23	3.33
60	4	324	6.91	2.42	6.79	6.86	6.04	3.27	8.16	3.38	5.45	3.33
60	4	324	6.87	2.34	6.52	5.42	5.78	3.28	7.92	3.47	5.67	3.13
60	4	324	7.02	2.70	6.58	5.34	5.19	3.28	7.51	3.37	5.13	3.46

ANNEX D. Results of a 24 month long-term stability study for individual PFASs (expressed in ng/L) in IRMM-428. Six independent replicates (two units analysed in triplicate) per time-point were measured.

L-PFOS T= -20 ºC

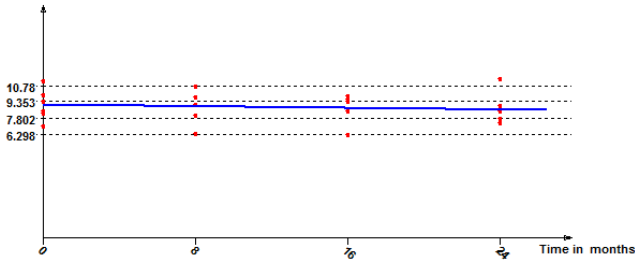
	t(month)			
Replicate	0	8	16	24
1	9.36	10.78	9.35	8.99
2	7.13	9.80	9.93	8.50
3	10.02	8.11	8.53	7.43
4	11.27	9.79	6.30	7.37
5	8.28	6.36	9.62	11.45
6	8.50	9.09	9.40	7.80

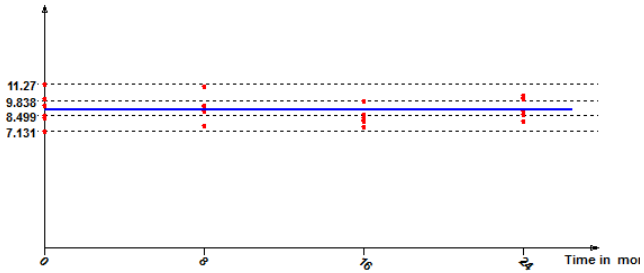
L-PFOS T= 4 ºC

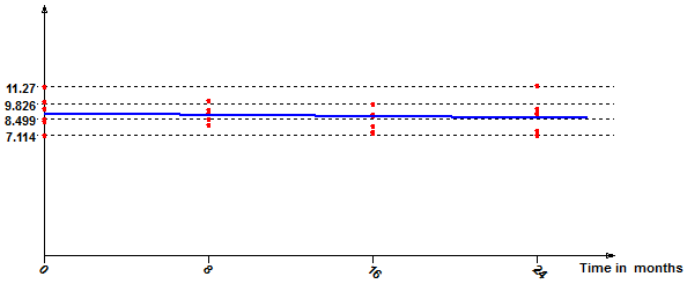
	t(month)			
Replicate	0	8	16	24
1	9.36	7.63	9.84	10.08
2	7.13	11.12	7.98	7.99
3	10.02	9.11	8.32	8.59
4	11.27	9.39	7.51	8.89
5	8.28	9.34	8.63	10.30
6	8.50	8.87	8.06	10.04

L-PFOS T= 18 ºC

	t(month)			
Replicate	0	8	16	24
1	9.36	8.53	7.45	7.47
2	7.13	9.00	8.77	8.89
3	10.02	9.26	9.83	9.37
4	11.27	8.98	7.32	7.11
5	8.28	10.07	7.91	8.98
6	8.50	7.99	8.85	11.43





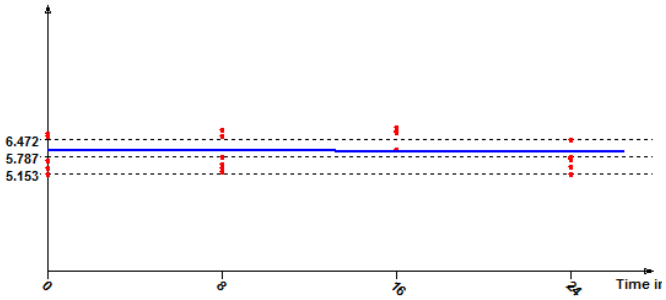


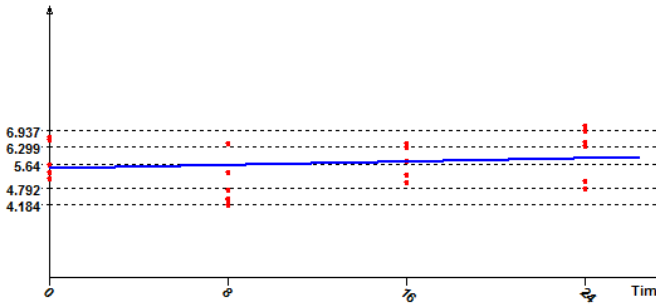
ANNEX D. Results of a 24 month long-term stability study for individual PFASs (expressed in ng/L) in IRMM-428. Six independent replicates per time-point were measured.

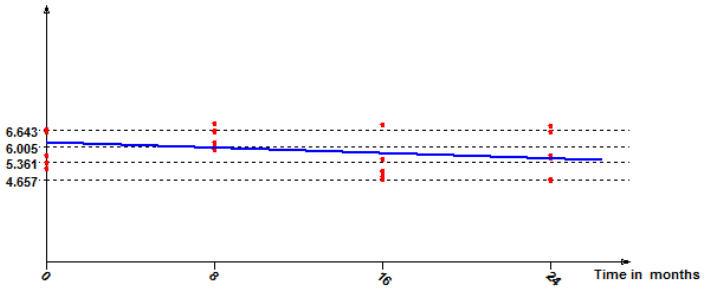
PFOA	T= -20 ºC			
	t(month)			
Replicate	0	8	16	24
1	6.69	6.61	6.07	5.79
2	5.36	6.82	6.75	5.16
3	6.57	5.40	6.78	5.75
4	6.67	5.82	6.09	5.41
5	5.64	5.50	6.76	6.47
6	5.15	5.26	6.94	5.69

PFOA	T= 4 ºC			
	t(month)			
Replicate	0	8	16	24
1	6.69	5.38	6.31	6.50
2	5.36	4.18	5.28	5.05
3	6.57	4.72	6.30	6.94
4	6.67	6.46	6.47	6.36
5	5.64	4.37	5.02	7.09
6	5.15	4.40	5.81	4.79

PFOA	T= 18 ºC			
	t(month)			
Replicate	0	8	16	24
1	6.69	6.58	4.73	4.66
2	5.36	6.15	5.06	6.59
3	6.57	6.94	6.88	5.68
4	6.67	6.64	5.50	4.71
5	5.64	6.00	5.01	5.59
6	5.15	5.90	4.83	6.83







ANNEX D. Results of a 24 month long-term stability study for individual PFASs (expressed in ng/L) in IRMM-428. Six independent replicates per time-point were measured.

PFDA **T = -20 ºC**

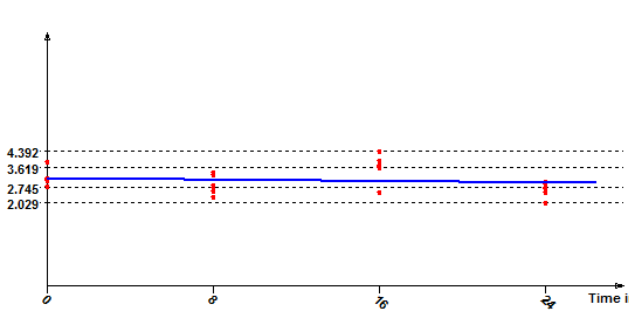
	t(month)			
Replicate	0	8	16	24
1	2.76	2.57	3.73	2.74
2	2.79	3.40	3.69	2.03
3	3.16	2.31	2.52	2.65
4	3.90	3.29	3.94	2.98
5	3.05	2.71	3.62	2.85
6	3.12	2.85	4.39	2.53

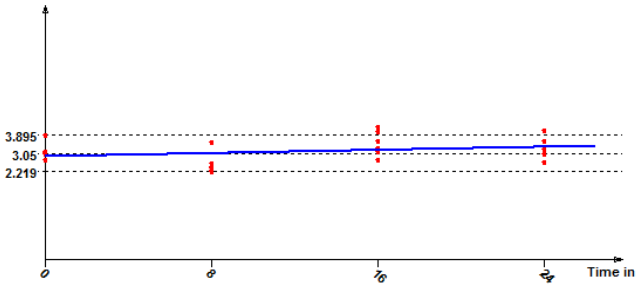
PFDA **T = 4 ºC**

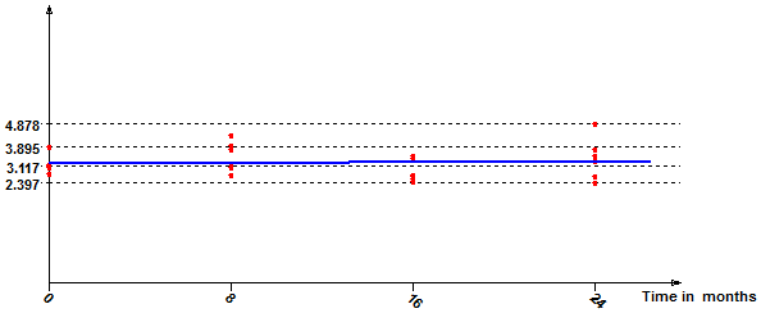
	t(month)			
Replicate	0	8	16	24
1	2.76	2.22	4.04	3.06
2	2.79	2.61	3.17	2.68
3	3.16	2.47	3.30	3.25
4	3.90	3.56	4.29	3.62
5	3.05	2.38	2.77	4.12
6	3.12	2.31	3.65	3.34

PFDA **T = 18 ºC**

	t(month)			
Replicate	0	8	16	24
1	2.76	2.70	2.67	4.88
2	2.79	3.04	2.55	3.52
3	3.16	3.79	3.53	3.32
4	3.90	4.35	2.74	2.67
5	3.05	3.96	3.42	2.40
6	3.12	3.18	2.43	3.80





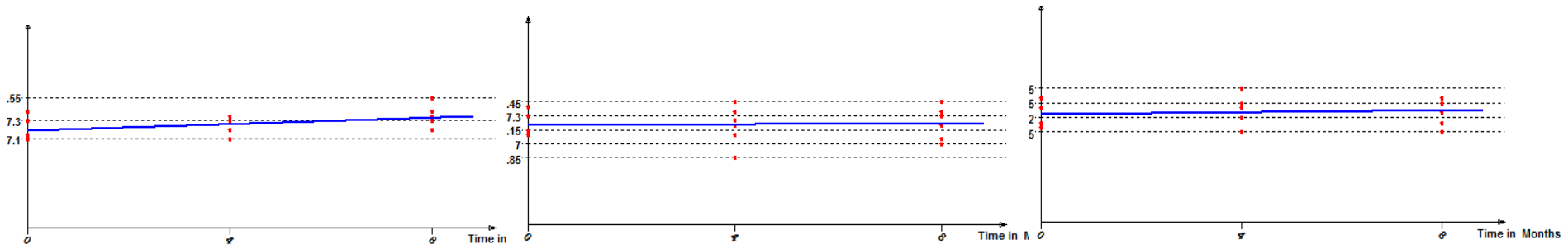


ANNEX D. Results of a 24 month long-term stability study for individual PFASs (expressed in ng/L) in IRMM-428. Six independent replicates per time-point were measured. Exceptionally data from an 8 months stability study is presented for PFBA.

PFBA	T= -20 ºC		
	t(month)		
Replicate	0	4	8
1	7.4	7.2	7.3
2	7.15	7.3	7.55
3	7.15	7.2	7.2
4	7.3	7.35	7.35
5	7.15	7.2	7.3
6	7.1	7.1	7.4

PFBA	T=4 ºC		
	t(month)		
Replicate	0	4	8
1	7.4	6.85	7.2
2	7.15	7.45	7.45
3	7.15	7.2	7.05
4	7.3	7.25	7.35
5	7.15	7.35	7.3
6	7.1	7.1	7

PFBA	T=18 ºC		
	t(month)		
Replicate	0	4	8
1	7.4	7.05	7.15
2	7.15	7.3	7.4
3	7.15	7.5	7.05
4	7.3	7.5	7.35
5	7.15	7.2	7.25
6	7.1	7.35	7.25



ANNEX D. Results of a 24 month long-term stability study for individual PFASs (expressed in ng/L) in IRMM-428. Six independent replicates per time-point were measured.

PFHxA T= -20 ºC

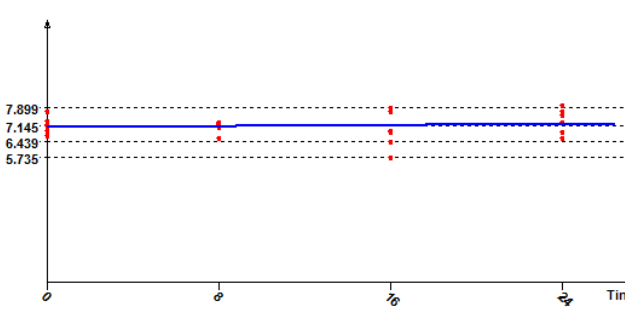
	t(month)			
Replicate	0	8	16	24
1	7.11	7.14	7.90	7.58
2	6.70	7.27	6.44	7.26
3	7.77	7.20	6.87	6.83
4	7.34	7.09	7.77	7.78
5	6.92	7.17	5.74	8.02
6	7.15	6.60	6.93	6.61

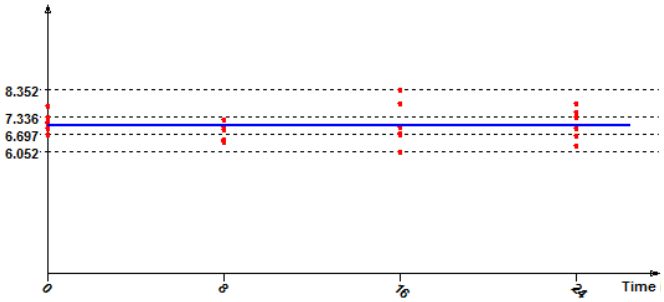
PFHxA T = 4 ºC

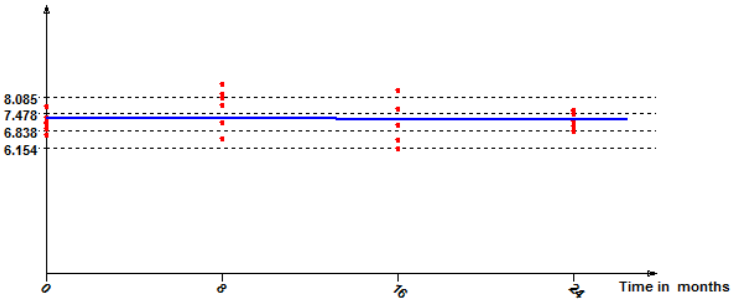
	t(month)			
Replicate	0	8	16	24
1	7.11	7.24	8.35	7.35
2	6.70	6.86	6.72	7.53
3	7.77	6.51	6.98	6.27
4	7.34	6.96	7.83	7.87
5	6.92	6.44	6.70	6.91
6	7.15	6.39	6.05	6.64

PFHxA T = 18 ºC

	t(month)			
Replicate	0	8	16	24
1	7.11	8.61	6.15	6.84
2	6.70	7.14	7.69	7.58
3	7.77	6.53	8.36	7.63
4	7.34	8.23	7.06	7.02
5	6.92	8.09	6.51	7.48
6	7.15	7.81	6.51	7.19







ANNEX D. Results of a 24 month long-term stability study for individual PFASs (expressed in ng/L) in IRMM-428. Six independent replicates per time-point were measured.

PFHpA T= -20 ºC

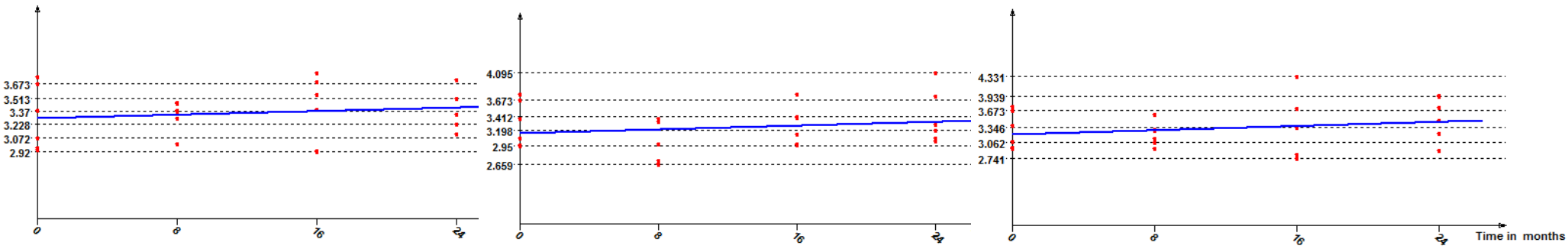
	t(month)			
Replicate	0	8	16	24
1	3.67	3.29	2.92	3.51
2	2.97	3.45	3.69	3.12
3	3.38	3.38	3.39	3.23
4	3.75	3.46	2.94	3.72
5	3.07	3.37	3.55	3.51
6	2.95	3.01	3.79	3.34

PFHpA T=4 ºC

	t(month)			
Replicate	0	8	16	24
1	3.67	3.34	3.39	4.09
2	2.97	2.68	3.13	3.07
3	3.38	2.99	3.76	3.28
4	3.75	3.38	2.99	3.20
5	3.07	2.66	2.96	3.73
6	2.95	2.73	3.41	3.02

PFHpA T=18 ºC

	t(month)			
Replicate	0	8	16	24
1	3.67	3.31	2.82	3.23
2	2.97	3.29	3.35	3.73
3	3.38	3.59	4.33	3.47
4	3.75	3.14	3.70	2.91
5	3.07	2.94	2.76	3.94
6	2.95	3.06	2.74	3.96

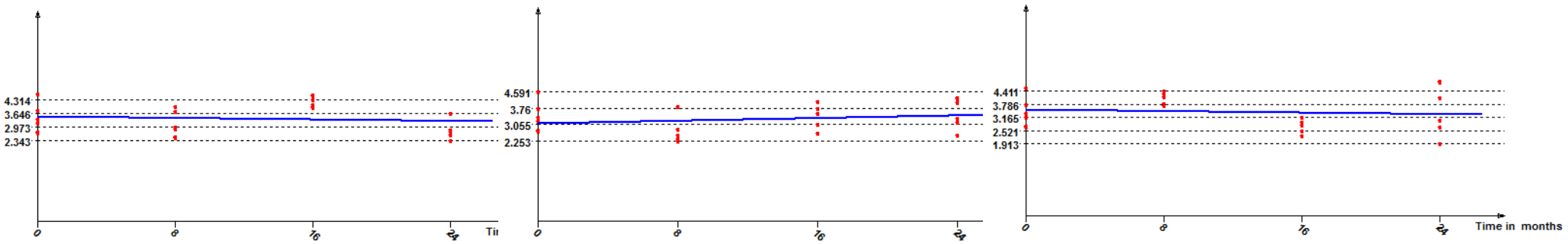


ANNEX D. Results of a 24 month long-term stability study for individual PFASs (expressed in ng/L) in IRMM-428. Six independent replicates per time-point were measured.

PFNA	T= -20 ºC			
	t(month)			
Replicate	0	8	16	24
1	4.59	2.97	4.07	2.86
2	2.71	3.99	4.47	2.34
3	3.19	2.54	4.31	2.74
4	3.38	2.49	3.98	2.60
5	2.76	3.72	3.92	3.65
6	3.79	2.91	4.52	2.61

PFNA	T=4 ºC			
	t(month)			
Replicate	0	8	16	24
1	4.59	2.79	3.54	3.30
2	2.71	2.25	3.05	2.54
3	3.19	2.34	3.55	4.27
4	3.38	3.89	3.76	4.06
5	2.76	2.32	2.62	4.30
6	3.79	2.53	4.10	3.13

PFNA	T=18 ºC			
	t(month)			
Replicate	0	8	16	24
1	4.59	3.83	2.96	4.11
2	2.71	3.71	2.52	2.71
3	3.19	4.17	3.17	3.05
4	3.38	4.28	2.81	1.91
5	2.76	4.41	2.53	4.84
6	3.79	3.82	2.31	4.90

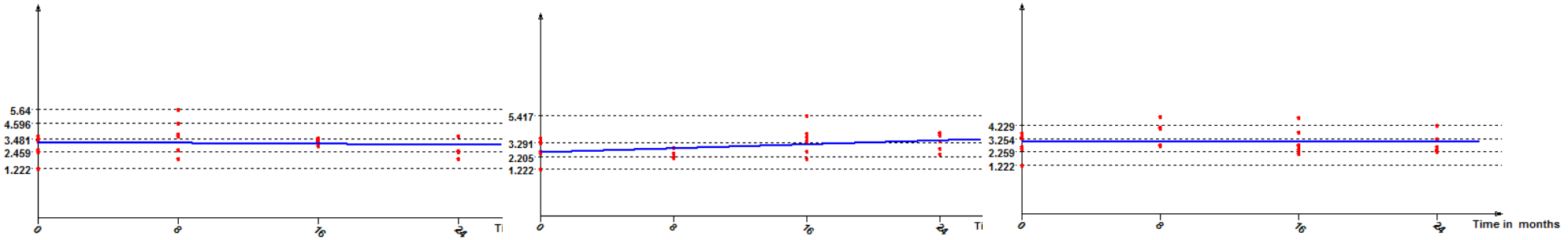


ANNEX D. Results of a 24 month long-term stability study for individual PFASs (expressed in ng/L) in IRMM-428. Six independent replicates per time-point were measured.

PFUnA	T= -20 ºC			
	t(month)			
Replicate	0	8	16	24
1	1.22	3.66	3.35	3.65
2	2.46	3.78	2.96	2.60
3	3.41	2.00	3.21	1.98
4	3.69	4.60	3.55	2.49
5	2.61	5.64	3.48	3.66
6	3.29	2.64	3.29	1.98

PFUnA	T=4 ºC			
	t(month)			
Replicate	0	8	16	24
1	1.22	2.92	3.42	3.88
2	2.46	2.20	3.77	2.86
3	3.41	2.90	2.65	3.42
4	3.69	2.50	5.42	4.01
5	2.61	2.21	2.07	4.08
6	3.29	2.11	4.02	2.39

PFUnA	T=18 ºC			
	t(month)			
Replicate	0	8	16	24
1	1.22	2.76	3.77	4.23
2	2.46	2.74	2.41	2.26
3	3.41	4.13	4.84	2.34
4	3.69	4.01	2.80	2.44
5	2.61	4.88	2.74	2.63
6	3.29	2.81	2.15	3.25



ANNEX D. Results of a 24 month long-term stability study for individual PFASs (expressed in ng/L) in IRMM-428. Six independent replicates per time-point were measured.

PFBS T= -20 ºC

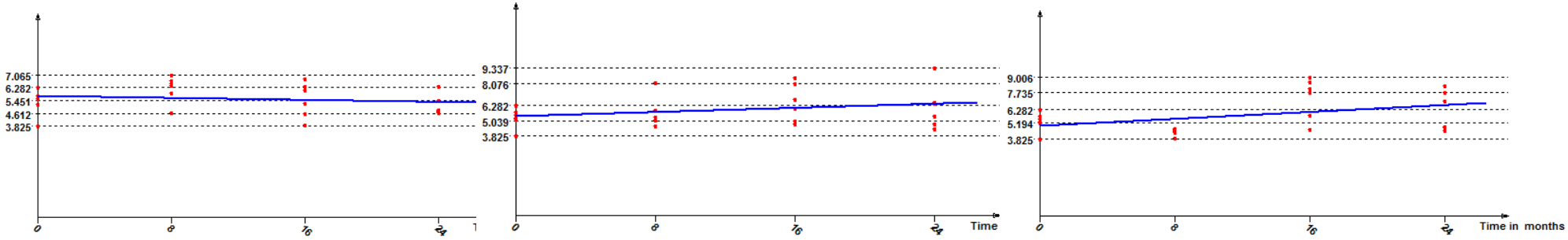
	t(month)			
Replicate	0	8	16	24
1	5.75	4.68	6.11	4.73
2	5.25	5.96	3.90	4.81
3	3.83	6.40	5.25	5.45
4	5.56	6.56	6.38	6.38
5	5.19	7.06	4.61	4.70
6	6.28	6.73	6.84	4.88

PFBS T=4 ºC

	t(month)			
Replicate	0	8	16	24
1	5.75	5.08	5.04	5.46
2	5.25	4.63	8.08	4.40
3	3.83	8.19	4.81	6.46
4	5.56	5.92	6.07	4.82
5	5.19	5.40	6.83	6.57
6	6.28	5.11	8.53	9.34

PFBS T=18 ºC

	t(month)			
Replicate	0	8	16	24
1	5.75	4.59	8.07	8.28
2	5.25	3.93	4.65	4.81
3	3.83	4.43	8.62	4.86
4	5.56	4.67	5.83	4.59
5	5.19	4.47	9.01	7.79
6	6.28	4.70	7.73	7.05

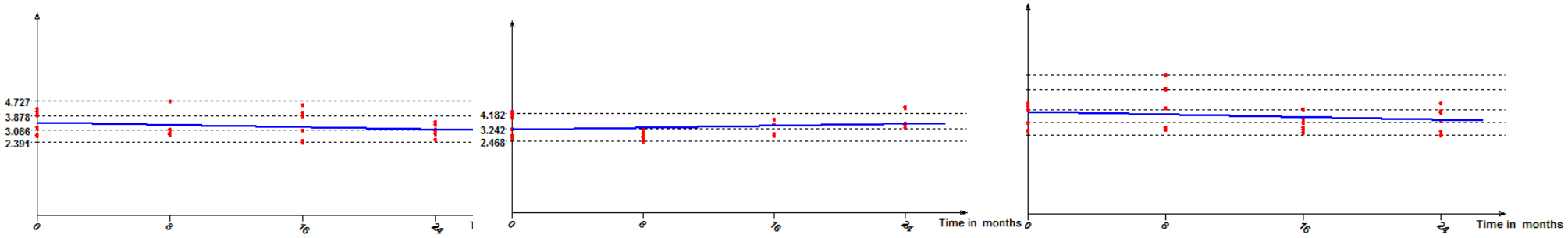


ANNEX D. Results of a 24 month long-term stability study for individual PFASs (expressed in ng/L) in IRMM-428. Six independent replicates per time-point were measured.

PFHxS	T= -20 ºC			
	t(month)			
Replicate	0	8	16	24
1	3.95	3.14	2.50	3.05
2	3.24	2.84	3.09	3.58
3	4.31	2.93	2.39	3.14
4	4.18	3.09	4.10	2.55
5	2.83	4.73	4.51	3.41
6	2.75	2.89	3.88	2.86

PFHxS	T=4 ºC			
	t(month)			
Replicate	0	8	16	24
1	3.95	2.47	3.53	3.31
2	3.24	2.98	3.84	4.57
3	4.31	3.22	2.99	3.61
4	4.18	2.72	2.97	3.47
5	2.83	2.81	2.87	4.60
6	2.75	2.71	3.85	3.32

PFHxS	T=18 ºC			
	t(month)			
Replicate	0	8	16	24
1	3.95	2.99	2.87	3.33
2	3.24	4.04	3.46	3.90
3	4.31	5.10	4.02	3.80
4	4.18	2.87	2.73	2.54
5	2.83	5.09	2.98	4.32
6	2.75	5.85	3.26	2.77

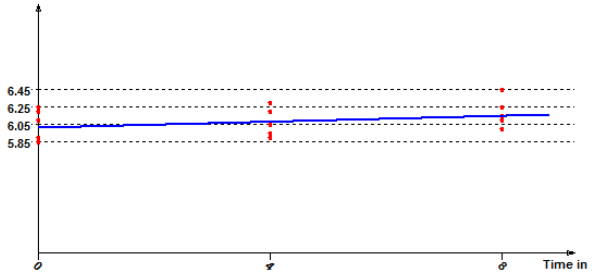


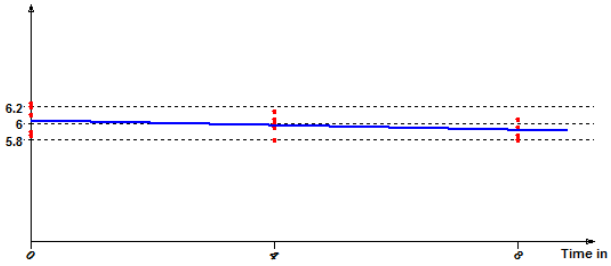
ANNEX D. Results of a 24 month long-term stability study for individual PFASs (expressed in ng/L) in IRMM-428. Six independent replicates per time-point were measured. Exceptionally data from an 8 months stability study is presented for PFPeA.

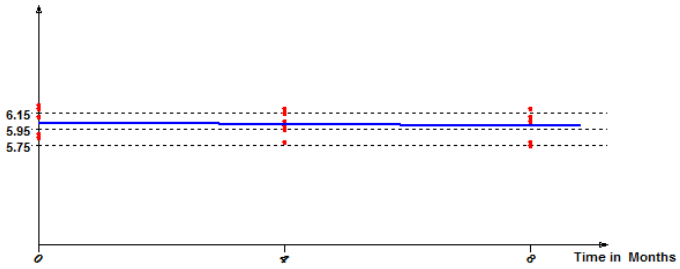
PFPeA	T= -20 ºC		
	t(month)		
Replicate	0	4	8
1	5.85	5.95	6.00
2	6.10	6.20	6.10
3	6.20	5.90	6.00
4	6.25	6.05	6.45
5	5.85	6.30	6.15
6	5.90	5.90	6.25

PFPeA	T=4 ºC		
	t(month)		
Replicate	0	4	8
1	5.85	5.95	5.80
2	6.10	6.15	5.95
3	6.20	5.95	5.80
4	6.25	6.05	6.05
5	5.85	5.80	6.05
6	5.90	6.00	5.85

PFPeA	T=18 ºC		
	t(month)		
Replicate	0	4	8
1	5.85	6.00	6.05
2	6.10	5.80	6.05
3	6.20	6.15	5.80
4	6.25	6.20	6.10
5	5.85	6.05	6.20
6	5.90	5.95	5.75







ANNEX E. Conditions and results from the qNMR analysis of neat crystalline PFOA and PFDA used for preparation of PFOA and PFDA common calibrant solutions. Purity results, expressed as mass fractions, correspond to average values obtained for 4 replicate analyses.

Compound	Purity [mg/g]	u (k=2)	H_2O [mol/mol F-DA]
PFOA	952.1	1.7	1
PFDA	951.6	1.7	1.8

qNMR	Instrumental conditions	Units
Spectrometer	Bruker DMX 400	
Wave frequency	376.47	MHz
Spectral width	15015	Hz
Relaxation delay	40	s
Data acquisition time	8.7	s
Number of scans	256	
Excitation 90 19F pulse	12.7	µs
Solvent	DMSO-d6	
Quantity standard	4-F Benzoic acid (4-BZA)	
T	333.2	K

ANNEX F. Table 1. Analytical LC-MS/MS methods applied for characterisation of PFASs in water by participating laboratories during the certification campaign of IRMM-428. All laboratories applied negative electrospray ionisation (ESI).

			Guard column		LC column				
Lab. code	sample intake	Extraction and Clean-up	Brand and type	Column dimensions	Brand and type	Column dimensions	Mobile phase	Quantification	Calibration type
L01	100 ml	SPE (Oasis-WAX eluted 2% NH4OH) SPE washed with 40/60 MeOH/H2O	C18	n.r.	ACE 3 C-18-300	C18, 2.1 x 150 mm, 3 µm	A: 95/5 MeOH/H_2O B: 40/60 MeOH/H_2O	n.r.	n.r.
L02	130 ml	SPE (Oasis-WAX conditioned 0.1 % NH4OH in MeOH and H2O, washed with 40/60 MeOH/H2O and eluted 2% NH4OH in MeOH) Extract filtered through acrodisc filter 0.2 µm	C18	n.r.	ACE	C18, 2.1 x 150 mm	A: 95/5 MeOH/H_2O B: 40/60 MeOH/H_2O	Internal standard	calibration curve
L03	100 ml	SPE (Oasis-WAX conditioned MeOH with 0.1 % ammonia, MeOH and H_2O. Washed with $AcNH_4$ (pH 4) and eluted with MeOH and 0.1 % ammonia in MeOH)	n.r.	n.r.	HSS T3, Waters	C18, 2.1 x 100 mm, 1.8 µm	A: 5 mM ammonium acetate B: MeOH	n.r.	n.r.
L04	100 ml	SPE (Oasis-WAX conditioned 0.1 % Na_4OH in MeOH and H_2O, washed with $AcNH_4$, THF:MeOH MeOH/H_2O and eluted 0.1 % NH_4OH in MeOH) evaporate to dryness, reconstitution MeOH with IS and H_2O	Symmetry column C18	n.r.	ES industries (132211-FO)	FluoroSEP-RP Octyl, 2.1 x 150 mm, 5 µm	A: Ammonium formate 5mM in H_2O B: MeOH	Internal standard	calibration curve

L06	120 ml	SPE (C8 + quaternary amine) rinse with MeOH with 1 % 1-methyl piperidine (1-MP), MeOH and H_2O. Rinse with MeOH. Elution with 80:20 MeOH/acetonitrile with 2 % 1-MP	C18	n.r.	Acquity BEH C18	C18, 2.1 x 50 mm	A: H_2O:MeOH + 2mM NH_4OAc B: MeOH + 2mM NH_4OAc	Internal standard	calibration curve
L07	150 g	Adjust to pH=4, Oasis Wax SPE (150 mg)	Phenomenex Luna, 3u, C8(2), Mercury MS	4.0 x 20 mm	Phenomenex Luna, 5u, PFP(2)	Pentafluorophenyl, 3.0 x 150 mm, 5 µm, 100 Å		Internal standard	calibration curve
L08	100 ml	SPE WAX	yes	n.r.	Waters Acquity UPLC HSS 3T column	C18, 2.1 × 100 mm, 1,8 µm	A: 2 mM NH_4OAc in 90:10 methanol/water B: 2 mM methanolic NH_4OAc	Internal standard	calibration curve
L09	100 ml	Oasis Wax SPE	n.r.	n.r.	C18MS	C18, 2.1 x 100 mm, 3.5 µm		Internal standard	calibration curve
L10	100 ml	n.r.	Thermo Prism RP (50 mm x 2.1 mm, 5u)	n.r.	Betasil C18	C18, 2.1 x 100 mm, 5 µm	A: 2mM Aqueous Ammonium acetate, B: Acetonitrile	Internal standard	calibration curve
L11	100 g	Extraction and concentration with SPE cartridges WAX	C18	n.r.	Waters Acquity UPLC BEH Shield RP 18,	Shield RP18, 2.1 x 100 mm, 1.7 µm	95:5 water:MeOH + 2 mM ammonium acetate	Internal standard	calibration curve

L12	50 ml	SPE weak anion exchanger (conditioned 0.1 % NH_4OH in MeOH and H_2O, washed with H_2O, aceton/ACN with 1% formic acid, MeOH and eluted 0.1% NH_4OH in MeOH)	C18	n.r.	MN Nucleodur Sphinx RP	C18, 2.0 x 100 mm, 3 µm	A: Water (+ 2mM Ammonium acetate) B: Methanol (+ 0.05 % Acetic acid)	n.r.	n.r.

ANNEX F. Table 2. m/z transitions used by participating laboratories for quantification (in bold) and identification of PFASs in IRMM-428

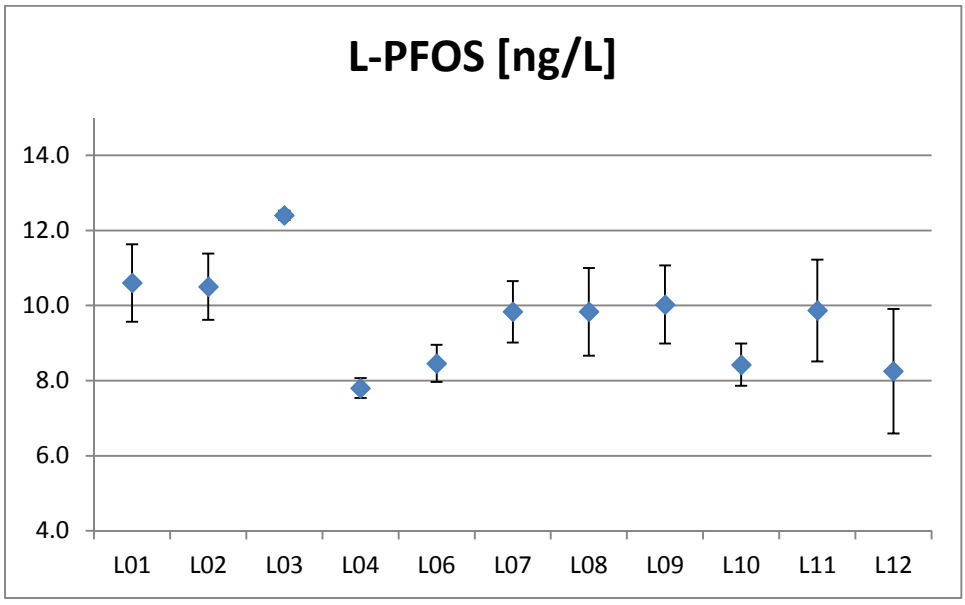
Laboratory code	L01	L02	L03	L04	L06	L07	L08	L09	L10	L11	L12
L-PFOS	499>80 499>99	**499>80** 499>99	**499>79.9** 499>98.9	**499>80** 499>99	**498.9>99** 498.9>80	**499>80** 499>99	**499>80** 499>99	499>80	499>80 499>99 499>130	**499>80** 499>99	**498.9>80** 498.9>98.9
PFDA	513>469 513>219	**513>469** 513>269	**513.1>469** 513.1>219	**513>468.9** 513>219	**513>469**	**513>469**	**513>469** 513>269	513>469	513>469 513>269 513>219	**513>469** 513>219	**513>468.8** 513>268.9
PFOA	413>369 413>169	**413>369** 413>169	**413>369** 413>169	**413>369** 413>219/169	**413>369**	**413>369** 413>169	**413>369** 413>169	**413>369**	413>369 413>219 413>169	**413>369** 413>169	**413>368.9** 413>168.9
FOSA							**498>78** 498>498				
PFBS	299>80 299>99	**299>80** 299>99	**299>80** 299>99	**299>80** **299>99**	**298.9>80**	**299>80** 299>99	**298>80** 298>99		299>80 299>99		
PFHxS	399>80 399>99	**399>80** 399>99	**398.8>79.9** 398.8>98.9	**399>80** 399>99	398.9>80	**399->80** 399->99	**398.9>80** 398.9>99		399>80 399>99		
PFHpS						**449>80** 449>99	**449>80** 449>99				
PFDS					**598.9>80**	**599>80** 599>99	**599>80** 599>99				
PFBA	213>169	**213>169**	**213>168.9**	**213>169** 213>213	**213>169**	**213>169**	**213>169**		213>169		
PFPeA	263>219	**263>219**	**263>219**		**263>219**	**263>219**	**263>219**				
PFHxA	313>269 313>119	**313>269** 313>119	**263>219**	**313>269** 313>119	**313>269**	**313>269**	**313>269** 313>119		313>269 313>119		
PFHpA	363>319 363>169	**363>319** 363>169	**363>319** 363>169	**363.1>319** 363.1>169.1	**363>319**	**369** **>319**	**363>319** 363>169		363>319 363>169		
PFNA	463>419 463>219	**463>419** 463>219	**463.1>419** 463>219	**463>419** 463>219	**463>419**	**463>419**	**463>419** 463>219		463>419 463>219 463>169		
PFUnDA	563>519 563>269	**563>519** 563>269	**563>519** 563.01>269	**562.9>518.9** 562.9>268.9	**563>519**	**565>520**	**563>519** 563>269				
PFDoDA		**613>569** 613>319	**613>569** 613.01>169	**613>568.9** 613>318.9	**613>569**	**613>569**	**613>569** 613>169				

PFTrA		**663>619** 663>369					**663>619** 663>169				
PFTeA		**713>669** 713>369					**713>669** 713>169				

ANNEX G. Characterisation data reported for PFASs in IRMM-428 by participating laboratories. Error bars in the graphs represent the standard deviation of the results for the individual laboratories.

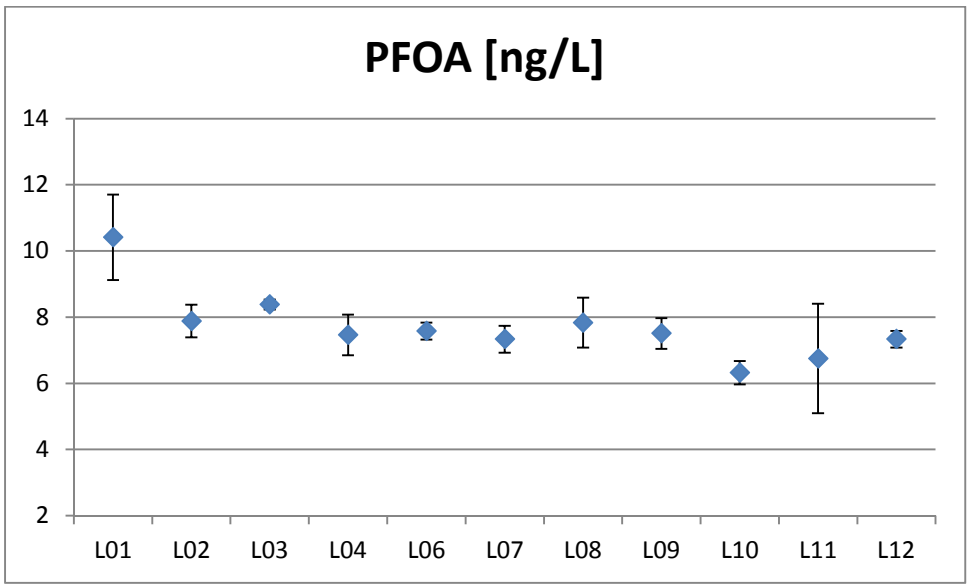
ANNEX G. Table 1. Analytical results of certification campaign for L-PFOS in IRMM-428. Error bars in the graphs represent the standard deviation of the results for the individual laboratories.

	L-PFOS (ng/L) in IRMM-428							
Lab code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	mean	standard deviation
L01	10.1	10.7	12.1	9	10.6	11.1	10.60	1.03
L02	9.1	11	11.3	11.3	9.9	10.4	10.50	0.88
L03	12.5	12.5	12.2	12.4	12.3	12.5	12.40	0.13
L04	7.98	7.8	7.96	7.39	7.59	8.1	7.80	0.27
L06	8.2	8.97	8.28	8.42	9.09	7.78	8.46	0.49
L07	10.5	11	10	9	9	9.5	9.83	0.82
L08	10	9	10	9	9	12	9.83	1.17
L09	11.5	9.25	9.8	11	9.84	8.77	10.03	1.04
L10	8.75	<LOQ	8.73	8.64	<LOQ	7.58	8.43	0.57
L11	12	11	8.8	9.1	9.7	8.6	9.87	1.36
L12	7	10	9	5.8	9.8	7.9	8.25	1.66



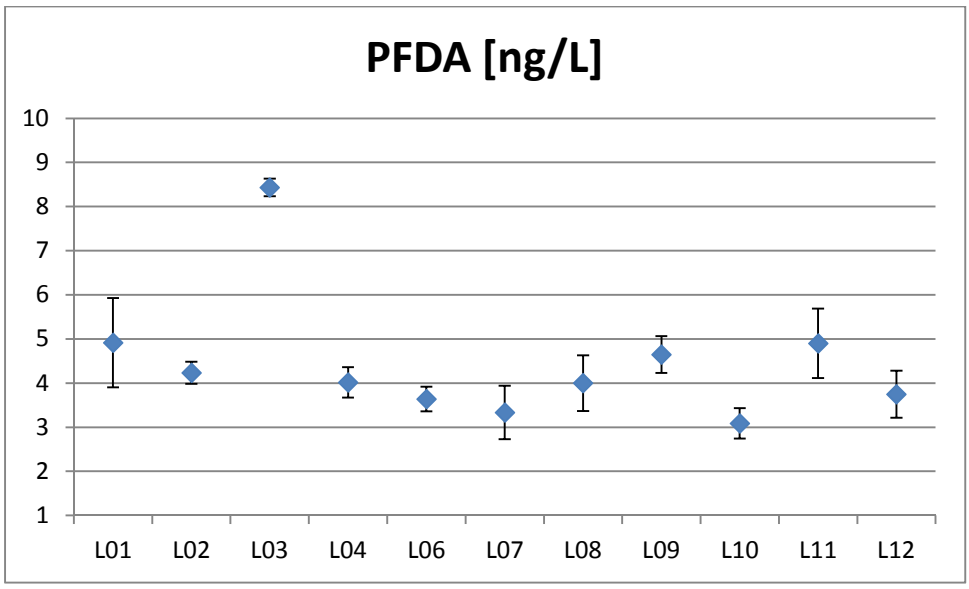
ANNEX G. Table 2. Analytical results of certification campaign for PFOA in IRMM-428. Error bars in the graphs represent the standard deviation of the results for the individual laboratories.

	PFOA (ng/L) in IRMM-428							
Lab code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	mean	standard deviation
L01	11.7	10.3	12.3	9.3	9.5	9.4	10.417	1.291
L02	7.3	8.5	8.3	8.1	7.7	7.4	7.883	0.492
L03	8.3	8.4	8.2	8.6	8.5	8.3	8.383	0.147
L04	7.84	7.4	7.8	7.8	6.26	7.68	7.463	0.611
L06	7.66	7.79	7.1	7.57	7.79	7.56	7.578	0.255
L07	8	7	7	7.5	7	7.5	7.33	0.41
L08	8	7	7	8	9	8	7.83	0.75
L09	7.69	7.88	6.97	7.45	8.09	6.97	7.51	0.47
L10	6.18	6.3	7.02	6.23	6.01	6.2	6.32	0.36
L11	6.8	9.5	7.3	4.5	5.9	6.5	6.75	1.66
L12	7.7	7.2	7.6	7.2	7.2	7.1	7.33	0.25



ANNEX G. Table 3. Analytical results of certification campaign for PFDA in IRMM-428. Error bars in the graphs represent the standard deviation of the results for the individual laboratories.

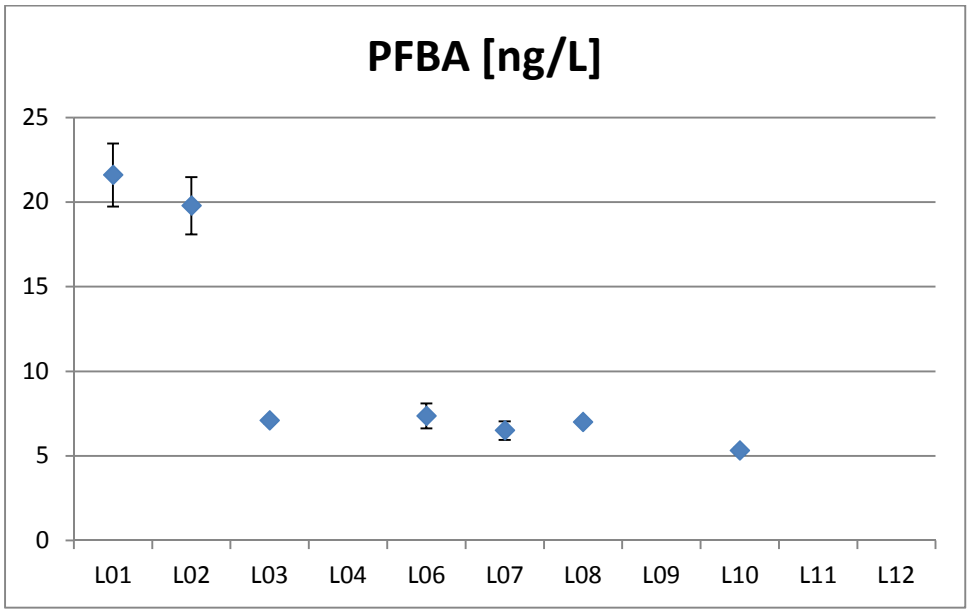
	PFDA (ng/L) in IRMM-428							
Lab code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	mean	standard deviation
L01	5.8	4.4	6.5	4	4.1	4.7	4.917	1.011
L02	3.9	4.3	4.4	4.6	4.1	4.1	4.233	0.25
L03	8.3	8.7	8.3	8.2	8.5	8.6	8.433	0.197
L04	4.02	3.65	4.32	4.01	3.63	4.48	4.018	0.344
L06	3.43	4.12	3.57	3.43	3.47	3.82	3.64	0.277
L07	3.5	4	4	3	3	2.5	3.33	0.61
L08	5	3	4	4	4	4	4.00	0.63
L09	4.55	4.97	4.36	4.56	5.3	4.15	4.65	0.42
L10	2.96	3.19	3.57	3.35	2.68	2.77	3.09	0.35
L11	5.9	5.7	4.5	4.9	4.6	3.8	4.90	0.79
L12	3.2	4.1	4.5	3.1	3.8	3.8	3.75	0.53



ANNEX G. Table 4. Analytical results of certification campaign for PFBA in IRMM-428. Error bars in the graphs represent the standard deviation of the results for the individual laboratories.

	PFBA (ng/L) in IRMM-428							
Lab code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	mean	standard deviation
L01	20.8	20.7	24.7	22.9	20.9	19.6	21.6	1.857
L02	21	18.3	20.2	22.2	17.7	19.3	19.783	1.689
L03	7.3	7.2	7	7.1	7.1	6.8	7.083	0.172
L04	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ		
L06	6.47	7.52	8.38	6.52	7.6	7.7	7.365	0.74
L07	6	6	7	7	6	7	6.5	0.548
L08	7	7	7	7	7	7	7	0
L09								
L10	5.25	5.33	5.08		5.57		5.308	0.204
L11								
L12								

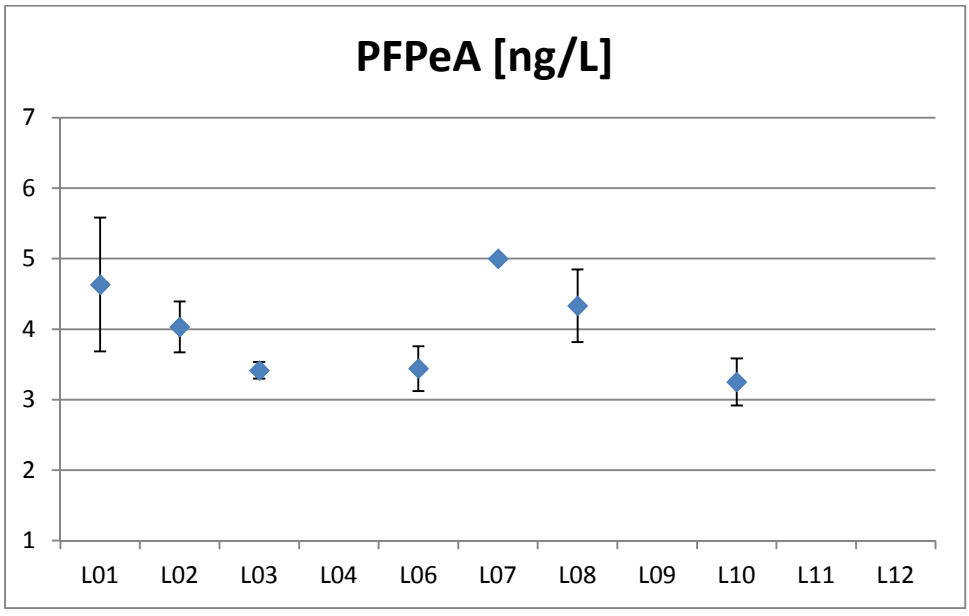
L04 results < LOQ



ANNEX G. Table 5. Analytical results of certification campaign for PFPeA in IRMM-428. Error bars in the graphs represent the standard deviation of the results for the individual laboratories.

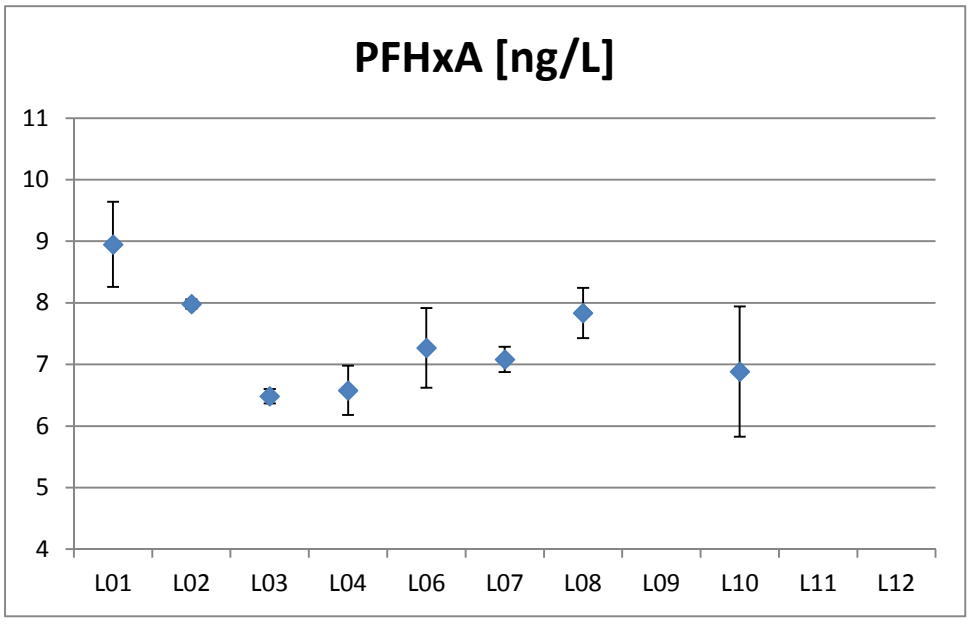
	PFPeA (ng/L) in IRMM-428							
Lab code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	mean	standard deviation
L01	4.4	3.8	6.3	4.4	3.8	5.1	4.633	0.948
L02	4.7	4	4	3.9	4	3.6	4.033	0.361
L03	3.4	3.3	3.5	3.4	3.6	3.3	3.417	0.117
L04	4.34	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ		
L06	3.47	3.36	3.19	3.24	3.32	4.06	3.44	0.319
L07	5	5	5	5	5	5	5	0
L08	4	4	5	4	5	4	4.333	0.516
L09								
L10	2.69	3.38	3.28	3.12	3.7	3.34	3.252	0.334
L11								
L12								

L04 excluded from evaluation since 5 out of six values were reported as below LOQ



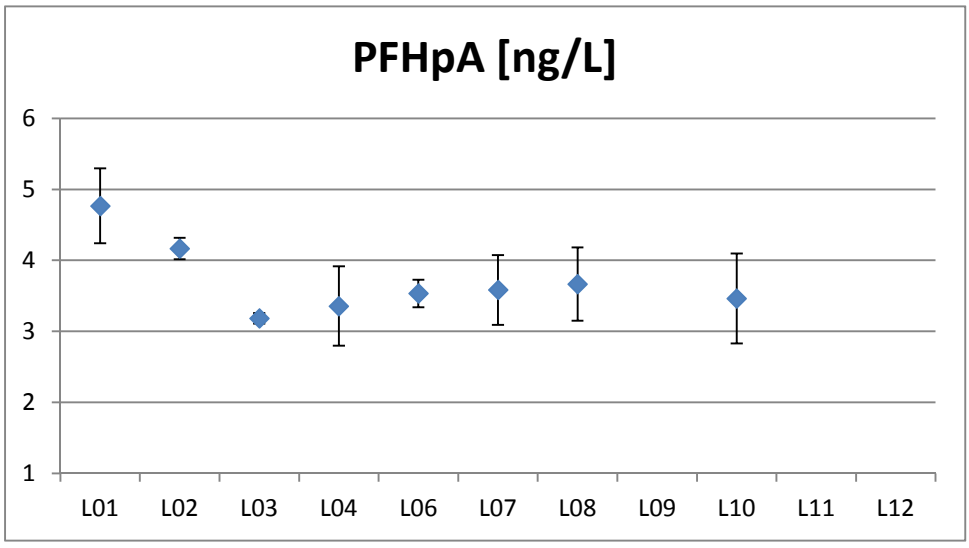
ANNEX G. Table 6. Analytical results of certification campaign for PFHxA in IRMM-428. Error bars in the graphs represent the standard deviation of the results for the individual laboratories.

	PFHxA (ng/L) in IRMM-428							
Lab code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	mean	standard deviation
L01	9.5	8.4	10	9	8.2	8.6	8.95	0.692
L02	8.1	8	8	8	7.9	7.9	7.983	0.075
L03	6.4	6.4	6.5	6.5	6.4	6.7	6.483	0.117
L04	6.35	6.12	6.73	7.13	6.25	6.9	6.58	0.4
L06	6.42	7.34	7.89	6.64	7.29	8.03	7.268	0.646
L07	7	7	7	7.5	7	7	7.083	0.204
L08	8	7	8	8	8	8	7.833	0.408
L09								
L10	5.7	7.85	7.07	5.58	7	8.11	6.885	1.057
L11								
L12								



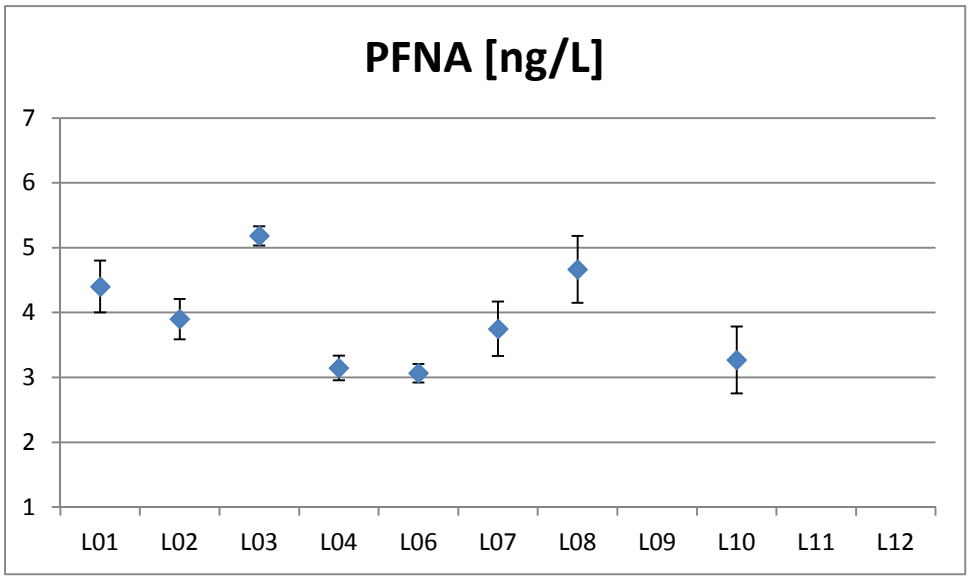
ANNEX G. Table 7. Analytical results of certification campaign for PFHpA in IRMM-428. Error bars in the graphs represent the standard deviation of the results for the individual laboratories.

	PFHpA (ng/L) in IRMM-428							
Lab code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	mean	standard deviation
L01	5.5	4.3	5.3	4.7	4.2	4.6	4.767	0.528
L02	4.1	4.1	4.3	4.4	4	4.1	4.167	0.151
L03	3.1	3.2	3.2	3.3	3.2	3.1	3.183	0.075
L04	3.34	2.67	4.36	3.42	3.09	3.26	3.357	0.559
L06	3.29	3.51	3.71	3.32	3.66	3.71	3.533	0.192
L07	4	3.5	3	4	4	3	3.583	0.492
L08	4	3	4	4	4	3	3.667	0.516
L09								
L10	3.68	2.98	3.66	3.84	2.45	4.18	3.465	0.633
L11								
L12								



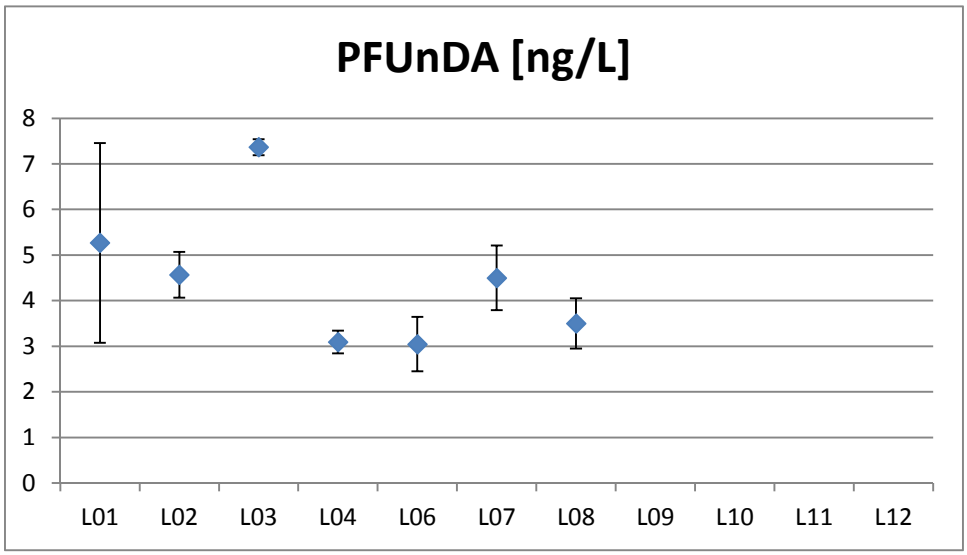
ANNEX G. Table 8. Analytical results of certification campaign for PFNA in IRMM-428. Error bars in the graphs represent the standard deviation of the results for the individual laboratories.

	PFNA (ng/L) in IRMM-428							
Lab code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	mean	standard deviation
L01	4.8	4.2	5	4	4.2	4.2	4.4	0.4
L02	3.6	4.1	3.8	4.4	3.9	3.6	3.9	0.31
L03	5.2	5.4	5.1	5.1	5.3	5	5.183	0.147
L04	3.41	3.34	2.98	2.95	3.09	3.11	3.147	0.189
L06	2.89	3.11	3	2.97	3.3	3.12	3.065	0.144
L07	4	4	3	4	4	3.5	3.75	0.418
L08	5	5	5	4	5	4	4.667	0.516
L09								
L10	3.89	2.83	2.74	3.62	2.85	3.69	3.27	0.517
L11								
L12								



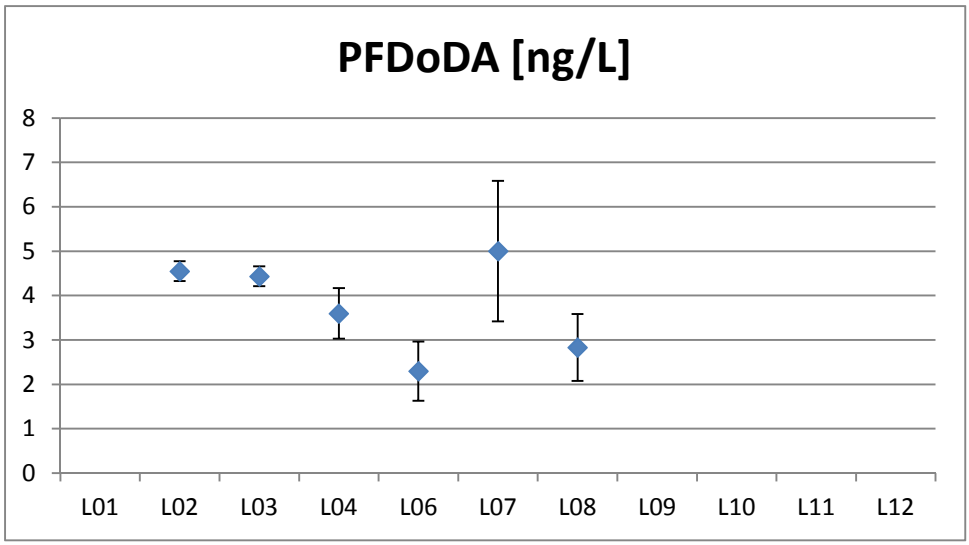
ANNEX G. Table 9. Analytical results of certification campaign for PFUnDA in IRMM-428. Error bars in the graphs represent the standard deviation of the results for the individual laboratories.

	PFUnDA (ng/L) in IRMM-428							
Lab code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	mean	standard deviation
L01	5.4	4.3	9.4	3	4.4	5.1	5.267	2.189
L02	4.4	4.6	4.2	5.5	4.6	4.1	4.567	0.501
L03	7.6	7.5	7.4	7.3	7.1	7.3	7.367	0.175
L04	3.42	3.08	2.72	3.15	3.09		3.092	0.25
L06	2.75	3.23	3.72	2.56	2.32	3.7	3.047	0.594
L07	4.5	5.5	5	3.5	4.5	4	4.5	0.707
L08	4	3	3	3	4	4	3.5	0.548
L09								
L10								
L11								
L12								



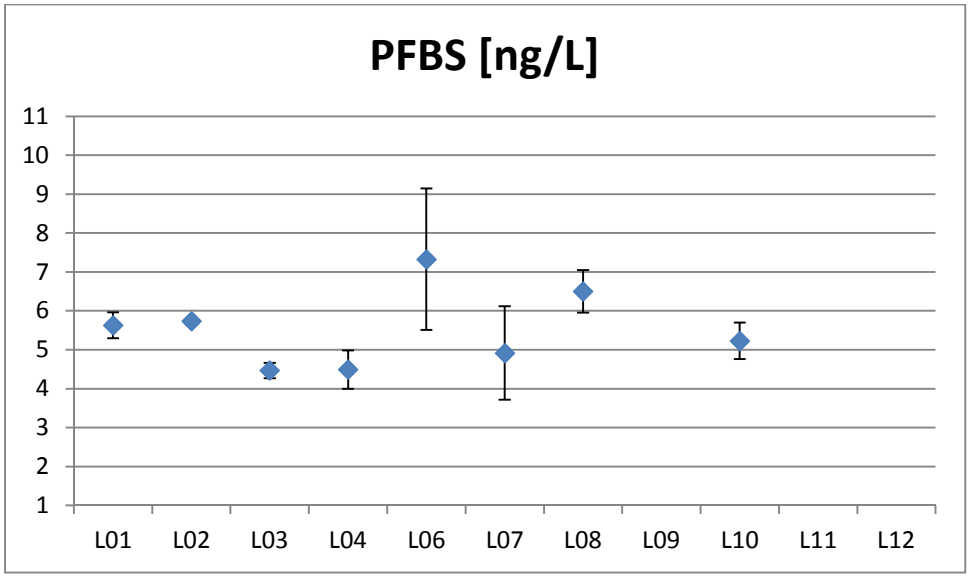
ANNEX G. Table 10. Analytical results of certification campaign for PFDoDA in IRMM-428. Error bars in the graphs represent the standard deviation of the results for the individual laboratories.

	PFDoDA (ng/L) in IRMM-428							
Lab code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	mean	standard deviation
L01								
L02	4.1	4.6	4.6	4.7	4.7	4.6	4.55	0.226
L03	4.7	4.2	4.6	4.6	4.2	4.3	4.433	0.225
L04	4.5	3.96	2.98	3.55	3.52	3.07	3.597	0.568
L06	2.02	2.26	3.49	2.36	1.46	2.2	2.298	0.666
L07	6	6	3	6.5	5.5	3	5	1.581
L08	3	3	2	4	3	2	2.833	0.753
L09								
L10								
L11								
L12								



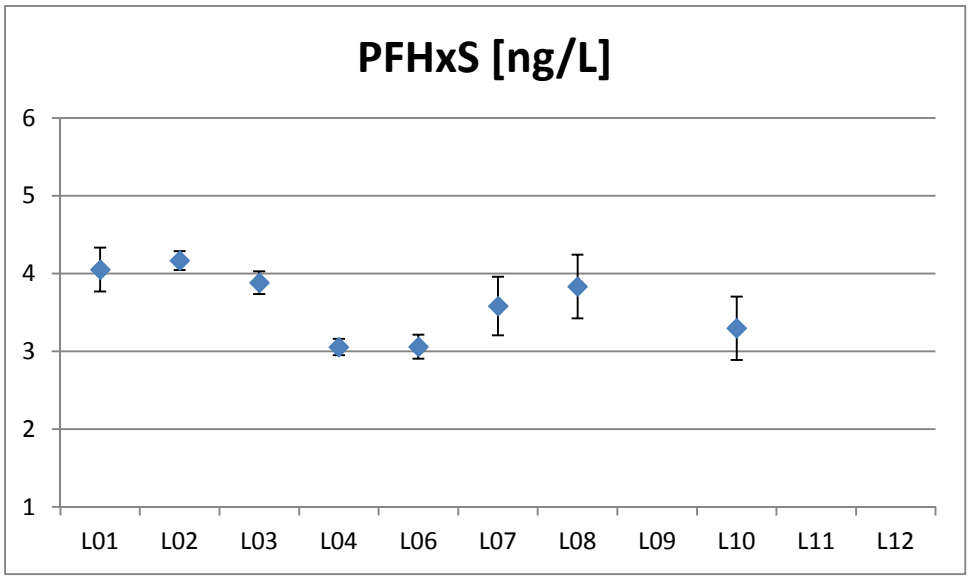
ANNEX G. Table 11. Analytical results of certification campaign for PFBS in IRMM-428. Error bars in the graphs represent the standard deviation of the results for the individual laboratories.

	PFBS (ng/L) in IRMM-428							
Lab code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	mean	standard deviation
L01	5.5	5.5	6.3	5.6	5.5	5.4	5.633	0.333
L02	5.6	5.7	5.8	5.7	5.8	5.8	5.733	0.082
L03	4.7	4.6	4.6	4.4	4.2	4.3	4.467	0.197
L04	4.07	4.88	5.14	3.86	4.31	4.68	4.49	0.493
L06	5.87	6.71	9.62	5.55	6.63	9.59	7.328	1.818
L07	5	6	3	5.5	6	4	4.917	1.201
L08	7	6	6	6	7	7	6.5	0.548
L09								
L10	5.16		5.54	4.59		5.62	5.228	0.47
L11								
L12								



ANNEX G. Table 12. Analytical results of certification campaign for PFHxS in IRMM-428. Error bars in the graphs represent the standard deviation of the results for the individual laboratories.

	PFHxS (ng/L) in IRMM-428							
Lab code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	mean	standard deviation
L01	4.1	4.2	4.5	3.9	3.9	3.7	4.05	0.281
L02	4	4.2	4.3	4.3	4.1	4.1	4.167	0.121
L03	4.1	3.8	4	3.8	3.7	3.9	3.883	0.147
L04	3.06	3.21	3.07	2.94	2.94	3.11	3.055	0.104
L06	3.08	2.85	3.22	3.15	3.17	2.89	3.06	0.154
L07	4	3.5	3.5	3.5	4	3	3.583	0.376
L08	4	4	4	3	4	4	3.833	0.408
L09								
L10	3.24	3.05	3.76	3.29	2.7	3.74	3.297	0.408
L11								
L12								



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Authors: Marta Dabrio Ramos, Ike van der Veen, Jean Charoud-Got, Hakan Emteborg, Jana Weiss, Heinz Schimmel

Luxembourg: Publications Office of the European Union

2015 – 73 pp. – 21.0 x 29.7 cm

ISBN 978-92-79-51037-3

ISSN 1831-9424

doi: 10.2787/736752

LA-NA-27430-EN-N

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ISBN 978-92-79-51037-3